# Design, synthesis and properties of a degradable polyurethane scaffold for meniscus regeneration

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Longitudinal lesions in menisci are among the most frequent orthopedic problems of the knee. Repair by simple techniques is only limited to the vascular part of the meniscus. For repair of the avascular part of the meniscus a scaffold, which will assist the body in the formation of new meniscus cell tissue, might be applicable. In this study a biomedical segmented polyurethane with poly(ε-caprolactone) as soft segment and 1,4-butanediisocyanate and 1,4-butanediol as uniform hard segments has been synthesised. The material has a micro phase separated morphology and excellent mechanical properties. A porous scaffold was prepared via a combination of liquid–liquid phase separation and salt leaching. The foams prepared combined a very high interconnectivity and porosity with the desired compression modulus. After six months of implantation in the knees of beagles full ingrowth with cells was obtained and it was found that meniscus like tissue had been formed in the scaffold. Moreover, compression behaviour appeared to be comparable to native meniscus tissue.


## Introduction

Due to the increase in sporting activities in recent years there has been a major increase in meniscal injuries. In most cases partial or complete menisectomy has to be applied, but it has been found that the meniscus can lose its functionality even when small parts of the meniscus are removed. For this reason it is preferred to preserve as much from the meniscal tissue as possible, however, repair of the knee joint meniscus would be more desirable.

In previous publications [1–4] it has been shown that it is possible to repair longitudinal meniscal lesions or replace the meniscus utilising degradable porous polymer scaffolds. Although the results seemed to be promising in these cases, the polyurethane used for meniscus replacement is known to release carcinogenic compounds upon degradation [5]. To avoid this problem a degradable polyurethane based on poly(ε-caprolactone) (PCL) and 1,4-butanediisocyanate/1,4-butanediol was developed [6, 7]. Upon degradation of the hard segment 1,4-butanediamine would be formed, which is a biocompatible compound [8]. The preparation of the poly(ε-caprolactone) polyol, as well as the chain extension step normally are catalysed by tin compounds. It is known that these catalysts also catalyse side reactions leading to branched and crosslinked polymers. During preparation of the foams high polymer concentrations are needed and good linear polymers would be preferred.

In this paper, a linear polyurethane based on 1,4-butanediisocyanate/1,4-butanediol and PCL with a short uniform hard segment was prepared. Foams with interconnected cells were prepared via a combination of thermally induced phase separation and salt leaching. The thermal and mechanical properties of the polymer and scaffold were studied. The scaffold was implanted into knees of dogs (beagles). Tissue ingrowth after six months of implantation was analysed and compared to that of the native meniscus. Moreover, the compression behaviour of the meniscal implants was determined and compared to that of native meniscus.

## Experimental

### Materials

1,4-Butanediol (BDO, Aldrich) was distilled from 3 Å molecular sieves. Prior to use 1,4-butanediisocyanate (BDI, Bayer) was distilled under reduced pressure using a short path distillation apparatus. ε-Caprolactone (Union Carbide) and dimethylsulfoxide (DMSO, Acros) were

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distilled from $CaH_2$ under reduced pressure. Dioxane (Merck) was distilled from sodium. Sodium chloride crystals (Merck) with the desired dimensions were obtained by sieving using NEN standard test sieves from Wilten (Etten-Leur, The Netherlands).

## Equipment

$^1$H-NMR spectra were recorded at room temperature using a 300 MHz Varian NMR apparatus. A Perkin-Elmer DSC-7 was used for thermal analysis, a heating rate of 10 °C/min. Molecular weights ($M_n$ and $M_w$) and molecular weight distribution ($M_w/M_n$) of the polyurethanes were determined by GPC measurements using dimethylformamide with 0.01 M LiBr as eluent on a Waters 600 Powerline system, equipped with two mixed-C Plgel 5-μ columns (Polymer Laboratories). Narrow polystyrene standards were used for calibration.

Tensile tests were performed using rectangular ($40 \times 2.2 \times 0.1$ mm) shaped specimens cut from solvent cast films. Tests were performed at 21 °C with a 100 N load cell and an extension rate of 10 mm/min using an Instron (4301) tensile tester.

Compression tests were performed on cubical foam samples of about $5 \times 5 \times 5$ mm at room temperature at a compression rate of 2 mm/min. The implanted samples were tested in phosphate-buffered saline.

A Jeol 6320 F field emission scanning electron microscope (FESEM) operating at a working distance of 11 mm, an acceleration voltage of 5 kV and a beam current of $1 \times 10^{-10}$ A was used for studying the pore structure of the porous materials.

## Synthesis

### *Poly(ε-caprolactone)diol*

A mixture of ε-caprolactone (29.10 g; 0.255 mol) and the initiator BDO (1.74 g; 0.019 mol) were reacted at 150 °C for seven days under an argon atmosphere to yield a polyester having a molecular weight of 1600 g/mol. $^1$H-NMR spectroscopy was used to verify the complete conversion of the polymerisation.

### *Endcapping of the poly(ε-caprolactone)diol*

A mixture of poly(ε-caprolactone)diol (28.07 g; 17.5 mmol) and a 12-fold excess of BDI (29.5 g; 0.21 mol) were reacted at 80 °C for 4 h under an argon atmosphere. The excess of the diisocyanate was removed using a short path distillation apparatus operating at 80 °C and $1 \times 10^{-2}$ mbar. Distillation was continued until a constant weight was reached.

### *Chain extension of the isocyanate terminated PCL*

The isocyanate terminated polyester (30.23 g; 16.1 mmol) was reacted with a slight excess of BDO (1.48 g; 16.4 mmol). After 72 h of reaction at 80 °C under an argon atmosphere the polymer was discharged from the flask.

### *Film casting*

The polymer was shredded and dissolved at 80 °C in dioxane (20 g/l). This solution was poured in a siliconised petri dish. The solvent was evaporated at 60 °C in 3 h. To remove the last traces of solvent the films were vacuum dried in a stove at 50 °C for 24 h. The films were stored at − 18 °C until use.

### *Foam preparation*

19.95 g of polymer was shredded and dissolved in 33.25 g DMSO at 80 °C. After 3 h 2.30 ml of water was added slowly under stirring. 156.6 g of NaCl crystals sieved to 150–355 μm and preheated to 130 °C, were added to the solution. This mixture was thoroughly mixed and divided over several moulds. The filled moulds were cooled in a freezer to − 18 °C after which the mixture was washed with a 20% ethanol in water solution. The foams were dried overnight in a vacuum stove at 37 °C.

## Surgery

Surgery was performed as described by Van Tienen *et al*. [9].

### *Compression*

After six months the meniscal implants were excised from the dog's knees and 4 mm biopsies were punched out of a specified region of the posterior horn of both the prosthesis and the native meniscus. These measurements were compared with the foam before implantation.

### *Histology*

Toluidine blue staining, to detect cartilaginous tissue formation and collagen type I and II labelling was performed as described before [10].

# Results and discussion

## Polymer synthesis

The polyurethane based on PCL was prepared in a two-step bulk polymerisation process using BDI and BDO. First, PCL was endcapped with an excess of BDI after which non-reacted BDI is distilled off. The complete removal of the unreacted BDI is important to ensure the well-defined hard block formation upon chain extension. Subsequently, this macrodiisocyanate was extended in the bulk at 80 °C for 72 h with a slight excess of BDO to ensure that the final polymer has a hydroxyl end group functionality minimising free isocyanate groups. The polymer was completely soluble in dioxane, chloroform and DMSO. The schematic representation of the synthesis is presented in Fig. 1. The obtained number average molecular weight was 156.1 kg/mol with a polydispersity of 2.2.

## Thermal and mechanical properties

The DSC thermogram of the film cast polymer shows a very low glass transition of − 56 °C suggesting a high

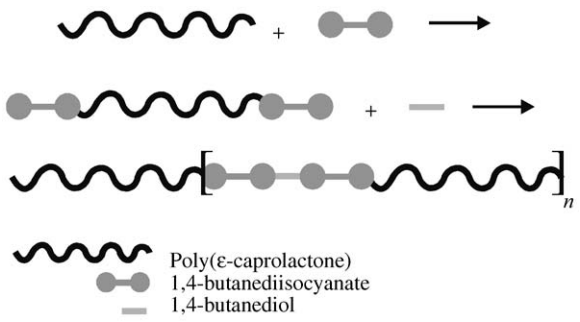


*Figure 1* Schematic representation of the two-step chain extension of PCL.

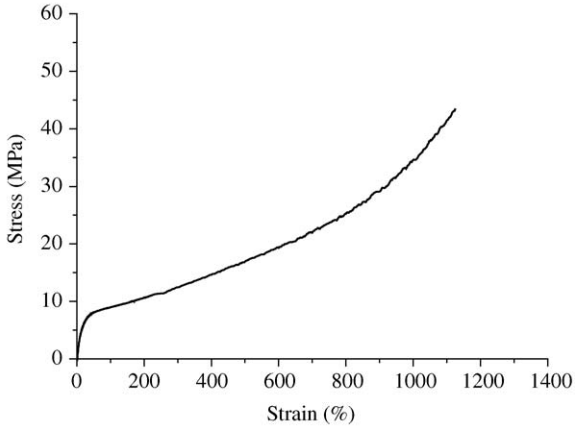


*Figure 2* Stress–strain curve of solvent cast film.

degree of phase separation and one melting point attributed to the hard segment. The absence of a second melting point suggests that there is no crystalline soft segment present. Crystalline soft segment generally shows a melting point around room temperature.

A typical stress–strain curve is presented in Fig. 2. The material showed a Young's modulus of 65 MPa and a strain at break of 1081%. The stress at break was found to be 44 MPa with an upturn effect due to strain-induced crystallisation.

## Foam

The foams were prepared by a combination of salt leaching and thermally induced phase separation. The polymer was dissolved in DMSO and a certain amount of water was added. DMSO is used in the medical field as a radical scavenger and generally accepted as biocompatible [11–13]. The use of nontoxic components during the production will prevent the presence of toxic materials in the end product. Water is added as a nonsolvent to decrease the solvent quality in order to induce liquid–liquid (L–L) phase separation upon cooling. Sodium chloride of a certain size is mixed into this solution at an elevated temperature. During cooling L–L phase separation took place resulting in a polymer-rich and a polymer-lean phase. After further cooling the solution gels preventing the salt from sagging out [14]. Further cooling results in freezing of the solvent.

The cold mixture was immersed in a nonsolvent bath (20% ethanol/water v/v) in which the polymer does not dissolve, but in which the DMSO, water and NaCl do dissolve. The foam is finally dried overnight in a vacuum stove. The SEM pictures of the foam were taken from a cutting plane showing a highly porous foam structure containing highly connected pores (Fig. 3). Fig. 3(b) shows a higher magnification in which an imprint of an NaCl crystal is still visible as a cubic-shaped hole.

Compressibility is of great importance for the performance of the implant, in the beginning of implantation as well as during the conversion into meniscus like tissue. Therefore, the compressive stress–strain behavior was determined. The compression modulus was determined via the stress–strain curve of the material before implantation (Fig. 5). From the slope of the curve at 20% compression a Young's modulus of 0.29 MPa was measured, which is a perfect value for a meniscus reconstruction material [3].

The first implanted menisci were hand made by cutting from a larger block of foam. As shown in Fig. 4, a meniscus-shaped foam can also be made via a suitable mould. Until now we have not found any limitation in the size of the foam.

## Implantation

The dogs regained their normal gait pattern 14 days after implantation. No infections were observed. All meniscectomised knees and knees with a meniscal implant were available for evaluation. After six months of implantation, the meniscal implants were completely infiltrated with tissue.

Especially in the role of stabilisation and alignment of

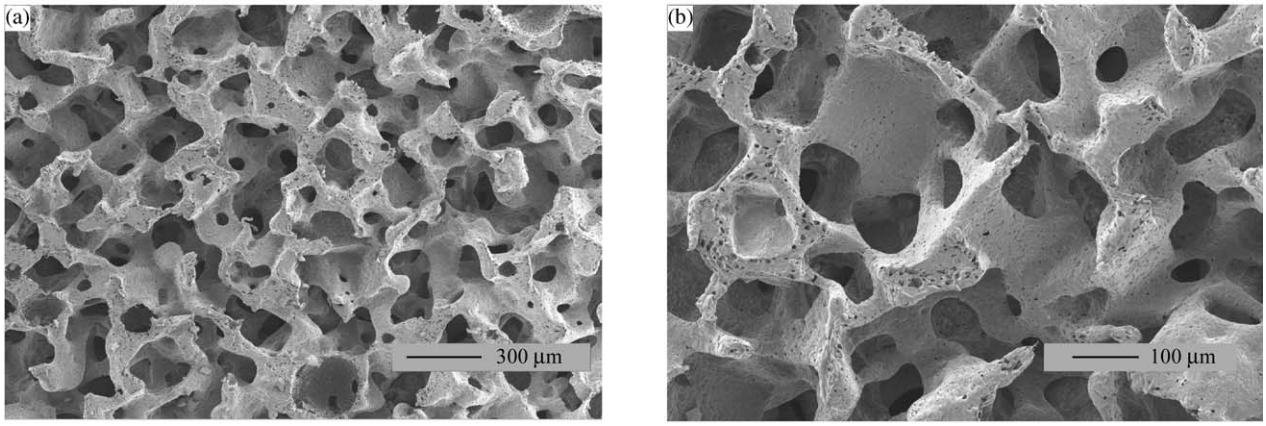


*Figure 3* SEM picture of a cross section of the foam.

*Figure 4* Meniscus-shaped scaffold.

the knee joint, a certain compression modulus is important to resist the high loading forces and to distribute these loads over a greater surface. Fig. 5 shows the compression curve of the scaffold before implantation, after six months of implantation and of the native meniscus. After six months the compression behaviour of the meniscal implant is approximating that of native meniscal tissue.

Meniscal fibrocartilage is described as a tissue containing fibrochondrocytes [15–17]. In the native meniscus collagen type I resembling more fibrous tissue is located in the periphery, whether collagen type II, resembling more cartilaginous tissue is located in the central rim of the native meniscus. In the present study staining with toluidine blue was performed to detect cartilaginous tissue and labelling with collagen type I and II antibodies was performed in order to detect type collagen I and II. It appeared that the areas of positive toluidine staining matched exactly with the areas of collagen type II antibody labelling. It was also found that the tissue distribution in both the meniscal implant and native meniscus was similar. In Fig. 6, the amounts of toluidine blue, collagen type I and II staining for the native meniscus and meniscal implant after six months of implantation is shown and appeared to be comparable. This is an indication that the tissue in the meniscal implant resembles meniscus-like tissue.

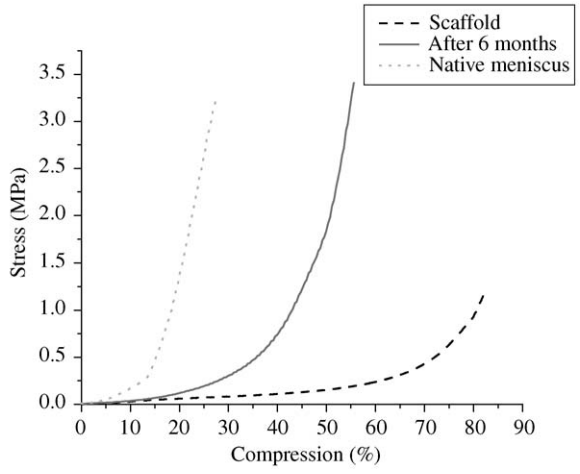


*Figure 5* Compression curve of scaffold before implantation, after six months of implantation and of the native meniscus.

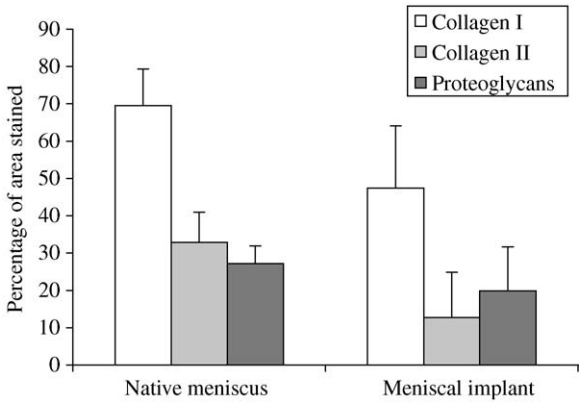


*Figure 6* Amount of toluidine blue, collagen type I and II staining for the native meniscus and meniscal implant after six months of implantation.

## Conclusions

The segmented polyurethane, based on poly(ε-caprolactone) diol, 1,4-butanediisocyanate and chain extended with butanediol, appeared to be easy processable polymer with excellent mechanical properties and good solubility. The chemical composition guarantees the release of fully biocompatible components upon biodegradation. Optimal foam porosity and interconnectivity was introduced via a combination of thermally induced phase separation and salt leaching. In this manner, a very porous structure was obtained with preservation of the desired mechanical properties. The combination of these properties makes the material very suitable as scaffold, especially as scaffold for meniscus replacement. The high interconnectivity allowed cells, nutrients and waste products to diffuse deeply in and out the structure allowing cells to proliferate even deep into the structure. It was also found that new, meniscus like tissue was formed and that the new tissue (in combination with the scaffold) shows comparable compression behavior compared to native meniscus tissue. From these observations we can conclude that the material is very promising as meniscus replacement scaffold.

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