PREPARATION OF POROUS BIODEGRADABLE POLYURETHANES FOR THE RECONSTRUCTION OF MENISCAL LESIONS


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Summary

Porous biodegradable poly(urethanes) for the reconstruction of the menisci have been prepared using two different combination of techniques: freeze-drying/salt-leaching and in-situ polymerization/salt-leaching. Using these methods, homogenous porous materials with a controllable and reproducible morphology can be prepared. The materials were made of three different poly(urethanes): a methylenediphenyldiisocyanate based polyurethane, a lysinediisocyanate based poly(urethane) and a poly(?-caprolactone) based poly(urethane). The compressive stress-strain behaviour of the Estane foams was determined. Foams made by the freeze-drying/salt-leaching technique implanted in dogs, showed healing and good ingrowth of fibrocartilaginous tissue.

Introduction

The menisci are very important mobile buffers which distribute the pressure of the upperleg over a larger area of the lower leg and increase the elasticity of the knee joint. The causes which can lead to damage or injury of the meniscus are multiple. It usually occurs when the knee is exposed to excessive demands. One of the most frequently performed orthopaedic operations is total or if possible partial meniscectomy. However, the results of meniscectomy in humans have been disappointing. Follow-up studies show satisfactory results in only 42% to 68% of the cases while Jorgensen et al. found radiographic changes of degeneration in 89% of the meniscectomy in athletes up to 14.5 years post operatively. Taking these results into account, reconstruction of the meniscus as an alternative has been gaining success.

It was already shown by King in 1936 that the regenerative response of the meniscus is poor. Healing is limited to lesions in the vascularized peripheral 10-25 percent of the meniscus. Lesions extending to the avascular central part of the meniscus do not heal, probably because they are not exposed to stimulatory factors normally present in blood, such as certain growth factors.

To overcome this problem, a connection between the lesion and the synovial lining can be made by implanting a porous polymer matrix into a defect. We have shown that repair of meniscus lesions in dogs is possible using a porous biodegradable PU/PLLA matrix. The materials were prepared by dipcoating. Ingrowth of fibrous, fibrocartilagenous tissue and vessels occurred over a considerable distance starting at the peripheral side of the implants and
signs of repair could be observed. Due to the positive results of this research we decided to undertake further studies.

For implantation, the porous materials have to fulfil a number of requirements. It has been found that optimum ingrowth of fibrocartilagenous tissue takes place if the pore size is in the range of 200-300 μm. These pores have to be highly interconnected. The tensile strength warrants stability and long term patency. The compressibility is of great importance because the nourishment of the cartilage comes in the flow of liquid brought on by the compression and relaxations arising from body movements. Compression movements also seem to stimulate fibroblasts to differentiate into chondrocytes. The porous implants should be compliant, act as a temporary biodegradable matrix scaffold for ingrowth of fibrocartilagenous tissue into implant and lesion, and leave a healed meniscus. Therefore the implant should be made of polymers which are designed to release only non-toxic degradation products. Nothing is known about the required degradation speed.

It is the purpose of this paper to describe different methods for making porous materials that fulfil these requirements. A modified salt-leaching combined/ or not combined, with a freeze-drying technique has been used to prepare low density polymer materials with controllable and reproducible structure and good mechanical properties. The foams are made of three different polymers: a methylenediphenyl-diisocyanate based polyurethane (Estane 5701-F1), a lysine diisocyanate based polyurethane and a poly(ε-caprolactone) based polyurethane. Some of these materials were implanted in dogs.

**Experimental**

**Material and Technique**

Estane 5701 F1 (Goodrich, Co, Brecksville, Ohio, USA) used for implantation was purified once by precipitation from a 5% (w/w) polymer solution in dimethylformamide (DMF) into a six fold volume of ice/water. Dioxane was distilled from sodium. Saccharose and NaCl crystals were sieved to 100-300 μm. NaCl was dried in a oven at 225 °C. Hexamethylene-diisocyanate (HDI) was vacuum-distilled before use. Dicumylperoxide (DCP) was recrystallised from methanol. Poly(ε-caprolactone)diol [(PCLdiol) M.W. = 1250] and Poly(ε-caprolactone)triol [(PCLtriol) M.W. = 900 (Aldrich Chemical Co.)] were azeotropically dried with benzene.
Poly(ε-caprolactone) diol (Mn = 1160; Janssen Chemical, Belgium) was dried under vacuum before use; 1,4-butanediol (Merck) was dried by distillation. The procedure for synthesizing ethyl 2,6-diisocyanatohexanoate (EDI) has been described in a previous publication\textsuperscript{13}. EDI was vacuum distilled prior to use. The materials were freeze-dried in a freeze-drying apparatus connected to a vacuum pump at 0.05 mBar.

PLLA fibres were prepared by a dry-spinning and a subsequent hot-drawing of the filaments method. The compressive stress-strain curves were determined using an Instron (4301) tensile tester (cross-head speed 2 mm/min, specimen height about 9 mm, 100 N load cell).

\textit{Lysine diisocyanate-based poly(urethane) (LDI-PU) Synthesis}

First 1.0 equivalent poly(ε-caprolactone) diol, 2.1 equivalents ethyl 2,6-diisocyanatohexanoate and 1.0 equivalent 1,4-butanediol were first mixed together and degassed at 90°C. Polymerizations were carried out under nitrogen atmosphere at 90-100°C for 3 hours, using 0.05-0.1 wt.-% stannous octoate (Sigma Chem. Corp. USA) as a catalyst. The resulting polymer was dissolved in DMF, precipitated into a tenfold excess of water and subsequently dried under vac. Yield 95-97%.

\textit{Foam Preparation}

The foams were made using two different methods. A combination of freeze-drying/salt-leaching techniques and a combination of in-situ polymerization/salt-leaching techniques were used.

\textit{Freeze-drying / Salt-leaching}

The polymer or prepolymer was dissolved in a solvent. Non-solvent was added to encourage phase separation. An amount of water soluble crystalline materials of variable particle size (NaCl and saccharose) was added. The mixture was poured into a mould and frozen at -15°C. Afterwards the mould was placed in a freeze-drying apparatus connected to a vacuum pump. The cosolvent was removed under reduced pressure (0.05 mBar). Due to endothermic evaporation of the cosolvent and to the high melting point of the solution, the samples remained frozen so that active cooling was not necessary. The saccharose and NaCl crystals were removed by washing the polymer/crystal mixture with water. The foams were
made of three different polymers: Estane, LDI-PU and a poly (?-caprolactone)-based poly(urethane) (PCL-PU).

**Estane foams:** Estane was dissolved in 1,4-dioxane. Water and c-hexane were added as non-solvents. The polymer solution was mixed with saccharose crystals (100-300 \(\mu\)m). The density of the foams was changed by varying the concentration of saccharose crystals.

**Crosslinked LDI-PU foams:** The polymer was dissolved in 1,4-dioxane. c-Hexane was added as a non-solvent. The polymer solution was mixed with DCP and NaCl crystals (100-300 \(\mu\)m). After freeze-drying the foam was crosslinked under nitrogen atmosphere for 3/4 hour at 150 °C. Afterwards the crosslinked porous polymer was extracted with chloroform (gel % = 90%)

**PCL-PU foam:** PCLdiol, PCLtriol and HDI were dissolved in 1,4-dioxane and mixed with NaCl crystals (50 wt.-% 50-90 \(\mu\)m and 50 wt.-% 250-300 \(\mu\)m). c-Hexane was added as a non-solvent. 0.1 wt.-% stannous octoate was added as a catalyst. After freeze-drying, the mixture was cured under nitrogen atmosphere at 60 °C for 48 hours and at 120 °C for 5 hours using 0.1 wt.-% stannous octoate as a catalyst. Afterwards the foam was extracted with chloroform.

**In-situ Polymerization / Salt-leaching**

The prepolymered used were diluted with solvent to encourage the homogeneity of the prepolymer with the crystals and was removed by evaporation before curing. The polymer/crystal mixture was washed with water to remove the crystals.

**PCL-PU foam:** PCLdiol, PCLtriol and HDI were dissolved in dichloromethane (37 wt.-% solution) and mixed with NaCl crystals (50 wt.-% 50-90 \(\mu\)m and 50 wt.-% 250-300 \(\mu\)m). 0.1 wt.-% stannous octoate was added as catalyst. Dichloromethane was removed by evaporation at 40 °C. The mixture was cured under nitrogen atmosphere at 60 °C for 48 hours and at 120 °C for 5 hours using 0.1 wt.-% stannous octoate as a catalyst.

**Compressibility**

Load-compression curves were obtained for a series of Estane foams varying in density \(\rho\) = 0.174 to 0.288 gram/cm\(^3\). All the data presented were obtained at room temperature and at a strain rate of 2 mm/min. The Young’s modulus and the modulus at 20 % compression of the foams were determined.
Characterizations

An Ubbelohde viscometer (type Oa) was used for the determination of the intrinsic viscosities of LDI-PU in chloroform at 25 °C.

Thermal analysis was performed with a Perkin Elmer DSC-7 (scan-speed 10 °C/min.). Characterisations of pore structure in the materials was performed using an ISI-DS scanning electron microscope.

Implant preparation

Estane foams used for implantation were prepared as described before. They were divided into two groups with different microporous structure due to different composition of the cosolvent: 1,4-dioxane/trioxane and 1,4-dioxane/c-hexane/water. 50% of the implants, made by freeze-drying an Estane solution in 1,4-dioxane/trioxane, were reinforced with PLLA fibres.

Implantation Technique

Foams used for implantation were made of Estane 5701-F1. A series of foams with different microporous structure were implanted. Large T-shaped lesions, occupying 30% of the meniscus were made in the midportion of the lateral menisci. The prostheses were sutured into place using 3.0 Dexon or mersilene sutures. After operation the dogs were allowed to walk as soon as possible. The follow-up period varied from 9-56 weeks and the menisci were studied both morphologically and histologically. Detailed information about the implantation technique has been described in a paper that will be published.

Results and discussion

We have showed that flexible porous polymer materials can be used for the reconstruction of meniscus lesions. For the ingrowth of fibrocartilagenous tissue in lesions and implant, the pores of the implant ought to be in the range of 200-300 μm and highly interconnected. In the past a dipcoating procedure was used and the resulting materials had a laminated structure. In these materials larger pores were dispersed in a more dense matrix with pore sizes up to about 60 μm. These materials induced showed complete repair and ingrowth of fibrocartilaginous tissue and vessels. The micro-porous matrix provides an excellent scaffold for capillary ingrowth. Although dipcoating is a suitable method, the resulting layered
structure was inhomogenous and a more controllable and reproducible technique would be preferable.

There are several other methods to produce flexible foams with an open-pore structure and can be divided into two groups. The first one utilizes blowing-agents\(^{17}\). In order to stabilize the structure and to control the pore sizes, additives (surface tension depressants) have to be added. The second one utilizes phase separation\(^{18}\). During cooling, polymerization of a polymer/prepolymer solution or adding non-solvent to a polymer solution, phase separation can take place. Removing the solvent results in a porous structure. When the phase-separated system is stable due to crystallization of the polymer out of the polymer-rich phase (eutectic-crystallization)\(^{19}\) or to the high Tg of the polymer-rich phase\(^{20}\), the solvent can be removed by evaporation without shrinkage of the porous structure. Otherwise the solvent has to be removed by sublimation (freeze-drying). The system is frozen and the polymer is not able to relax during solvent removal. To prevent the porous structure from shrinking and to avoid the need adding toxic components, the freeze-drying technique was chosen to prepare the porous materials.

*Freeze-drying process*

Freeze-drying of a polymer solution is a process where the solvent is removed by sublimation from the frozen material leaving a porous structure. The experimental process can be described as follows\(^{21}\): First the polymer is dissolved into a solvent system. The solution is then cooled until the solvent is frozen. Next the solvent is sublimated under vacuum resulting in a porous polymer structure.

The density of the resulting porous polymer is determined by the concentration of the polymer in solution. The morphology of the foam is determined by phase separation. Phase separation can be divided into liquid-liquid phase separation, which may occur prior to freezing of the solvent, and liquid-solid phase separation, which occurs when the solvent freezes. Adding a non-solvent to the solution may induce liquid-liquid phase separation\(^{22}\).

The morphology may be predicted from a phase diagram\(^{20}\). At low concentrations the polymer-rich phase will be dispersed in a dilute matrix. At high polymer concentrations the situation is inverted. During cooling the solution moves from a homogenous solution to a domain in which phase separation occurs. Phase separation will take place until the solvent is frozen. The final morphology is determined by whether liquid-liquid phase separation occurs before freezing or whether liquid-solid phase separation occurs.
Aubert et al.\textsuperscript{22} showed that if liquid-liquid phase separation occurs, the solution becomes cloudy before freezing and the resulting foam is isotropic and has small cell sizes (e.g. 10 μm). If only liquid-solid phase separation occurs then the resulting foam is anisotropic with a morphology depending on the crystal shape of the solvent.

The pore sizes can be regulated by the polymer concentration and the quench rate. An increase of the quench rate may increase the nucleation rate or lead to spinodal decomposition and decreases the time for phase separation resulting in smaller pores. Foams made by the freeze-drying technique have controllable and reproducible porous structure.

The systems used in this paper are poly(urethanes) dissolved in a mixture of 1,4-dioxane and water and/or c-hexane.

\textit{Foam Morphology}

Figure 1 shows a scanning electron micrograph of a freeze dried foam made from a 20 wt% polyesterurethane (Estane 5701-F1) solution in 1,4-dioxane/water (84/14 w/w). The solution was frozen at -15 °C. The structure is basically isotropic with pore sizes of <50 μm. It is due to a combination of liquid-liquid phase separation and liquid-solid phase separation. This agrees with the observation that the solution became cloudy before freezing.
Figure 2 shows a detailed scanning electron micrograph of the same foam. The pores are interconnected. The interconnected pores reach sizes in the range of 50 \( \mu \text{m} \) and are too small to fulfill the requirement for ingrowth of fibrocartilaginous tissue. The pore size can be controlled by varying the polymer concentration and the quenching rate. Although the pore sizes can be increased to about 200 \( \mu \text{m} \) by lowering the polymer concentration and/or the quenching rate such foams will not have sufficient strength for surgical applications.

To overcome this problem, a modified combination of freeze-drying and salt-leaching was used. The polymer solution was mixed with saccharose crystals (100-300 \( \mu \text{m} \)). After freeze-drying this mixture, the saccharose crystals were washed out in water leaving the macroporous structure.

Figure 3 shows a scanning electron micrograph of a freeze dried foam made from a 20 wt% PU (Estane 5701-F1) solution in 1,4-dioxane/c-hexane (79/21 w/w) mixed with saccharose crystals (100-300 \( \mu \text{m} \)). The weight ratio of the polymer solution to the saccharose crystals was 1:1. The structure contains large pores (100-300 \( \mu \text{m} \)) and small channel-like pores with diameters of <50 \( \mu \text{m} \). The large pores are due to the dissolution of the saccharose crystals and are connected with the small pores, which arise from the freeze-drying process.
Figure 4 shows a detailed scanning electron micrograph of the foam. Each large pore contains a skin so that the pores are not as well interconnected as they should be to fulfil the requirement of interconnectivity. Better interconnected structure was obtained by adding some water to the PU-cosolvent-sugar mixture. The sugar crystals partially dissolved in the water resulting in a better connection between large and small pores.

Figure 5 shows a scanning electron micrograph of a foam made by freeze-drying a 20 wt-% PU solution in 1,4-dioxane/c-hexane/water (78/18/4 w/w/w). Figure 6. Scanning electron micrograph of a foam made by freeze-drying a 20 wt-% solution in 1,4-dioxane/trioxane (50/50 w/w) mixed with saccharose crystals (100-300 μm).

Figure 4 shows a detailed scanning electron micrograph of the foam. Each large pore contains a skin so that the pores are not as well interconnected as they should be to fulfil the requirement of interconnectivity. Better interconnected structure was obtained by adding some water to the PU-cosolvent-sugar mixture. The sugar crystals partially dissolved in the water resulting in a better connection between large and small pores.

Figure 5 shows a scanning electron micrograph of a freeze dried foam made from a 20 wt% PU (Estane 5701-F1) solution in dioxane/c-hexane/water (78/18/4 w/w/w) mixed with sugar crystals (100-300 μm). The weight ratio of the polymer solution to the saccharose crystals was 46/54. The large pores are very open and contain no skin. This structure with its heavily interconnected large and small pores fulfil the requirements for ingrowth of fibrocartilagenous tissue and is suitable for surgical applications.
Figure 7. Scanning electron micrograph of a foam crosslinked with dicumylperoxide, made by freeze-drying a 20 wt.-% lysine diisocyanate-based PU solution in 1,4-dioxane/c-hexane (90/10 w/w) mixed with NaCl crystals (100-300 μm).

Figure 8. Scanning electron micrograph of a cross-linked foam made by a 37 wt.-% poly(ε-caprolactone)diol, poly(ε-caprolactone)triol and hexamethylene-diisocyanate solution in 1,4-dioxane mixed with NaCl crystals (50 wt.-% 50-90 μm and 50 wt.-% 250-300 μm).

Figure 9. Scanning electron micrograph of a foam made of a 37 wt.-% poly(ε-caprolactone)diol, poly(ε-caprolactone)triol and hexamethylene-diisocyanate solution in dichloromethane mixed with NaCl crystals. The solvent evaporized before polymerization.

The morphology can be altered by changing the solvent. Figure 6 shows a scanning electron micrograph of a freeze-dried foam made from a 20 wt.-% PU (Estane) solution in 1,4-dioxane/trioxane (50/50 w/w) mixed with NaCl crystals (100-300 μm). The weight ratio of the polymer solution to the salt crystals was 54:46. The microstructure contains needle-like pores.
due to the crystallization of the trioxane. Liquid-liquid phase separation did not occur before freezing. The microporous structure is not as porous as the microporous structure obtained by using 1,4-dioxane/c-hexane/water as cosolvent described previously. The freeze-drying/saltcasting process is applicable for any polymer for which appropriate solvents are available. Estane is a methylenediphenyldiisocyanate (MDI) based poly(urethane) which is converted into the toxic, mutagenic, carcinogenic diamine 4,4-dimethylenedianiline (MDA) after degradation\(^{24}\). This problem can be overcome by using aliphatic diisocyanate like hexamethylene diisocyanate (HDI). Bruin et al.\(^{13}\), used L-lysine based diisocyanate for the synthesis of biodegradable PU.

Figure 7 shows a scanning electron micrograph of a freeze-dried foam made from a 25 wt % LDI-PU (with an intrinsic viscosity of $[\eta] = 1.7$ dl/g) in 1,4-dioxane/c-hexane (90/10 w/w) mixed with NaCl crystals (100-300 \(\mu\)m). Before freeze-drying, the polymer was mixed with 10 % DCP. After freeze-drying, the foam was cross-linked for three quarters of an hour at 150\(^\circ\)C. Cross-linking of the polymer was necessary because the melting point of the polymer is 40 \(^\circ\)C due to the caprolactone fragments so the structure will loose its integrity after implantation. Part of the microstructure was lost due to melting of the polymer before cross-linking. The pores are highly interconnected and the foam will release non-toxic components when it degrades.

Another method to produce porous materials of polyurethanes is to polymerize in-situ the components in the presence of salt crystals. Before the polymerization, the solvent can either be removed by freeze-drying or by evaporation.

Figure 8 shows a scanning electron micrograph of a freeze dried foam made from a 37 wt.-% solution of 6 equivalent PCLdiol, 2 equivalent PCLtriol and 9 equivalent HDI in dioxane mixed with NaCl crystals (50 wt.-% 50-90 \(\mu\)m and 50 wt.-% 250-300 \(\mu\)m). Salt crystals with small diameters were added to increase the viscosity of the prepolymer solution/salt mixture so the crystals were restrained from sagging. The weight-ratio monomer/solution was 3:7. After freeze drying, the components were cured and the salt crystals were removed. The microstructure is not lost during the polymerisation. The macropores are highly interconnected with the micropores (<10 \(\mu\)m). During cooling liquid-liquid phase separation took place because the macrostructure is predominantly isotropic. This system can be used for any polymer in which the components are soluble in a suitable solvent. The components can easily be changed.
Figure 9 shows a scanning electron micrograph of a foam made from 37 wt.-% solution of 6 equivalent PCLdiol, 2 equivalent PCLtriol and 9 equivalent HDI in dichloromethane. The solvent evaporated before polymerization so the structure is due only to the salt crystals. In this structure, the pores are less interconnected than in the previous structures where a freeze-drying step was added. Increasing the salt concentration increases the porosity but decreases the strength of the foam drastically. Using this method, a highly interconnected structure is obtained by increasing the salt concentration so that the crystals touch each other but these foams are not suitable for surgical applications.

*Compressibility*

Because compressibility is of great importance for the performance of the implant, the compressive stress-strain behaviour was determined. The compressive stress-strain curve for a flexible foam exhibited linear elasticity at low stresses. It is followed by a long collapse plateau, due to the buckling of the walls between the pores, which is truncated by a regime of densification in which the stress rises steeply\(^2\).  

For a series of Estane foams of different densities, compression moduli (the Young's modulus and the modulus at 20% compression) were obtained. The modulus-density relationship is plotted in figure 10.

Doherty and coworkers\(^2\)^25 have shown that the change in physical properties with the density of urethane foams can be expressed as a straight lines on log-log plots. The logarithm of the relative density, \(? / ?_s\), is plotted against the logarithm of the relative modulus, \(E_f / E_s\), in figure 11 and fall on a linear curve with slopes for the Young's modulus and the modulus at 20% compression of 2.8 and 3.0 respectively. Where \(E_f\) and \(E_s\) are the Young's modulus (or modulus at 20% compression) of foamed and solid materials, respectively, and \(?_f\) and \(?_s\) are the respective densities.

Theoretically the relative Young's modulus for a foam is given by\(^2\)^26:

\[
E_f / E_s = (\rho_f / \rho_s)^2
\]
Figure 10: Relationship between modulus and density. (?): Young’s modulus, (o): Modulus at 20 % compression.

Figure 11: Relationship between the relative modulus $E/E_s$ and the relative density $\rho/\rho_s$. (?): Young’s modulus, (o): Modulus at 20 % compression.
This formula is based on the assumption that the structure remains the same when the density is changed. Because of the great importance of the macropores in implants, the density of the Estane foams is varied by changing the saccharose crystal concentration and so the macropores concentration increases with decreasing density. It is obvious that the modulus becomes more dependent on the density in this case.

*Implantation*

The results of the PU-PLLA and PU implants were comparable but there were considerable differences between the PU implants. The macroporous structure was similar in all the PU implants. The microporous structure was obtained using 1,4-dioxane/trioxane and 1,4-dioxane/c-hexane/water as cosolvents respectively. The later and most porous structure showed better results.

These implants showed a relative quick ingrowth. Fibrocartilagenous tissue turned out to be formed 15 weeks after implantation starting from the peripheral side of the meniscus. After 33 weeks about 50% of the polymer was degraded and 100% of the pores were filled with active chondroblasts and fibrocartilagenous matrix. This percentage fibrocartilagenous tissue was never seen in implants before.
Figure 12 shows a detail of an implant 33 weeks after implantation, including PU matrix (white coloured). The pores are totally filled with cartilaginous tissue (dark coloured) in which the active chondrocytes, that are making this tissue, are dispersed.

Conclusions
Salt casting in combination with freeze drying is a very good method for preparing reproducible porous polymer materials suitable for surgical application. The freeze-drying step is of great importance in order to accomplish a highly interconnected porous polymer materials with good mechanical properties.

Using a combination of in-situ polymerization and freeze-drying enable us to prepare porous networks of which the components could easily be varied to be prepared. Implants with a macroporous structure (100-300 μm) highly interconnected with a microporous structure (<50 μm) showed excellent ingrowth of fibrocartilagenous tissue. The porosity seems to affect the ingrowth speed of tissue into the implant.

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