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Heijkants, RGJC; Van Tienen, TG; De Groot, JH; Pennings, AJ; Buma, P; Veth, RPH; Schouten, AJ

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Preparation of a polyurethane scaffold for tissue engineering made by a combination of salt leaching and freeze-drying of dioxane

R. G. J. C. HEIJKANTS
Department of Polymer Chemistry, Faculty of Mathematics & Natural Sciences, State University of Groningen, Nijenborgh, 4,9747AG Groningen, The Netherlands

T. G. VAN TIENEN
Orthopaedic Research Laboratory, University Medical Center, P.O. Box 9101, 6500HB, Nijmegen, The Netherlands

J. H. DE GROOT, A. J. PENNINGS
Orteq B.V., Kamerlingheplein, 16,9712 TR Groningen, The Netherlands

P. BUMA, R. P. H. VETH
Orthopaedic Research Laboratory, University Medical Center, P.O. Box 9101, 6500HB, Nijmegen, The Netherlands

A. J. SCHOUTEN*
Department of Polymer Chemistry, Faculty of Mathematics & Natural Sciences, State University of Groningen, Nijenborgh, 4,9747AG Groningen, The Netherlands
E-mail: A.J.Schouten@rug.nl

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In the past several types of synthetic porous materials have been made as meniscus reconstruction materials. Most of these materials lacked a good combination between suitable mechanical properties and a high interconnectivity. In this work porous scaffolds were made from the polyurethane Estane 5701-F1 by freeze drying of a dioxane and water solution in combination with salt leaching. It was possible to obtain very porous scaffolds with a very high interconnectivity. Porosity, pore size and interconnectivity can be independently adjusted by varying the amount of water, porogen size and the amount of porogen used. The obtained compression moduli of the scaffolds were between 40 kPa and 400 kPa with a variation in porosity between 72 and 87%. These scaffolds are very suitable for the use as meniscus replacement materials.

1. Introduction

The menisci are C-shaped discs interposed between the femoral condyles and tibial plateau and have the function of shock absorption, stabilization and lubrication of the knee joint. Since the classical studies by Fairbank and King, it has been recognized that meniscectomy leads to articular cartilage degeneration [1, 2]. Today, the method of choice is partial meniscectomy to preserve as much meniscal tissue as possible. However, clinicians have noticed a high incidence of arthritic changes in the joint in mid- and long-term outcome studies of partial meniscectomy [3–5]. Reconstruction of the meniscus would be a good alternative and might preserve its functions and prevent damage to the cartilage. Several materials have been tried, but were not satisfying: Autologous materials [6, 7] possess poor initial mechanical characteristics, which makes long term fixation problematic. The calcifications that were found in case of perichondrium [8] and the adaptation to the shape of meniscus of patients also tends to be problematic [9]. An implant based on a donor meniscus seems at first glance an attractive option to replace a severely damaged meniscus [10]. In this case problems

*Author to whom all correspondence should be addressed.
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are related to availability, preservation techniques, possible transfer of diseases, individual shaping of the implant and possible immunological reactions to the implant [11]. To avoid the problems with an allograft implant, the group of Stone developed a meniscus implant based on collagen [12, 13]. Probably based on the complex suturing technique and the absence of knowledge on the long-term consequences for the articular cartilage, the support for this technique is very restricted, especially in Europe. Synthetic materials do not have the problems associated with inferior mechanical properties. Their properties can be adapted for the requirements of a specific application. More then one type of (degradable) biomaterial has been used for meniscus transplantation [14–16]. The problems with these types of implant were e.g., fragmentation of non-resorbable materials, which may induce macrophage or giant cell reactions [14], synovial irritation, cartilage softening [17]. Degradable scaffolds lack the combination of satisfying mechanical properties and a high interconnectivity [18]. It has been found that a fast infiltration of fibrous tissue into the polymer scaffold depends on the presence of interconnected macropores [19–21]. Furthermore, a higher stiffness (compression modulus) of the scaffold seemed to stimulate the differentiation of fibrous tissue into fibrocartilage [19]. Initially the tissue inside the scaffold needs to be protected from the forces that can occur in the knee, although mechanical stimulation of the cells is also necessary for the conversion into fibrocartilage tissue [22].

From literature it is known that the cell size of the scaffold is of great importance for the correct cell ingrowth [21, 23–25]. Moreover, interconnectivity, the connection between the pores in the scaffold, is very important since it has a decisive role in the diffusion of cells into the scaffold and the transport of nutrients and cellular waste products [16, 24–27]. Good interconnectivity facilitates diffusion and enhances the chance for cells deep inside the scaffold to survive. An accurate control over the interconnectivity independent of the pore size and overall porosity is thus necessary. De Groot *et al.* [18] studied foams and foam preparation methods based on Estane where the interconnectivity between the pores originating from the porogen was introduced by the crystallization of the solvent. However, this leads to systems with a high content of so-called micro pores. It appeared that this morphology was not suitable for cell ingrowth and cell sustainability on the long term.

In the present study the production of polymeric scaffolds that meet the above-mentioned requirements better are described. The different factors that influence the scaffold structure and the mechanical properties of the scaffold are described.

2. Experimental

2.1. Materials and methods

Estane (5701-F1) (BF Goodrich Chemical, Belgium) is a polyesterurethane with a hard segment based on 4,4-methylenediphenyldiisocyanate (MDI) and a soft segment based on poly(tetramethylene adipate). The polymer was purified once by precipitation from a 5 w/w% polymer solution in dimethylformamide (DMF) into a six-fold volume of R.O. water. The number average molar mass was 94.3 kg/mol with a polydispersity of 1.9. Dioxane (Merck) was distilled from sodium. Sucrose crystals (BDH) were sieved to the according size using NEN standard test sieves from Wilten (Etten-Leur, The Netherlands).

Molar masses ($\overline{M}_n$ and $\overline{M}_w$) were determined by GPC measurements using dimethylformamide with 0.01 M LiBr as eluents on a Waters 600 Powerline system, equipped with 2 mixed-C Plgel 5 μ columns (Polymer Laboratories) kept at 70°C. The data-analysis was done using conventional calibration with polystyrene standards. A Jeol 6320 F Field Emission Scanning Electron Microscope (FESEM) was used for studying the pore structure of the porous materials. It was operated at a working distance of 11 mm, an acceleration voltage of 5 kV and a beam current of 1×10^{-10} A. The specimens were made conductive with a 3 nm layer of gold using a Cressington Rotating Magnetron Sputter Coater operated at a working distance of 150 mm and a current of 20 mA.

Compression tests were performed on cubic shaped specimens of about $5 \times 5 \times 5$ mm cut manually from the scaffolds. The experiments were performed at 21°C with a 100 N load cell and a strain/compression rate of 2 mm/min using an Instron (4301) tensile tester. The compression modulus was determined at 20% compression.

2.2. Porosity calculation

The porosity of the scaffolds was determined by measuring the dimensions and the mass of the scaffold and calculated as follows:

$$p = \left(1 - \frac{m}{\rho_{\text{polymer}} \cdot V}\right) \cdot 100\%$$

where p is the porosity, m the mass of the scaffold, ρ_{polymer} is the density of the polymer and V is the volume of the scaffold.

2.3. Scaffold formation, a typical procedure

35.14 g Estane was dissolved in 45.3 g 1,4-dioxane at 80°C. 7.2 ml R.O. water was added as non-solvent to induce liquid-liquid phase separation upon cooling. Sucrose crystals (151 g sucrose), sieved to a size of 150–355 μm, were added and mixed in with a mechanical stirrer. The mixture was transferred into a mould and frozen at –18°C. Dioxane and water were removed through freeze-drying. Sucrose was removed by washing the polymer/sucrose mixture for 24 h with 1 l of water per gram of polymer. The scaffolds were dried in a vacuum stove at 37°C for 24 h.

3. Results and discussion

3.1. Polymer concentration, porogen and porosity

An optimal scaffold for meniscus regeneration should contain large pores for cell ingrowth, should have good mechanical (compression) properties and a high porosity. These three issues are in principle in conflict with each other. They were addressed separately as follows:

It has been found that optimal ingrowth of fibrocartilagenous tissue takes place if the pore size is in the range of 200–300 μm [18]. Therefore the scaffold should contain a maximal number of pores in that range, consequently as much sugar as possible should be mixed with the polymer solution. However, the upper limit here is determined by the processability of the final mixture and the compression modulus of the resulting foam.

A better processability might be obtained by decreasing the polymer concentration of the solution, but this leads to a relatively high content of micro pores, that decrease the compression modulus of the foam and do not contribute to the accessibility of the scaffold for cells and nutrient and waste diffusion. This was the case for the foams described by De Groot. There the foams had a similar size of the large pores in combination with a major amount of micro pores that could not be infiltrated with cells. Moreover, the interconnectivity was determined by the size and number of the micro pores [18]. A decrease in small pores in combination with a constant overall porosity and an increase in interconnectivity will increase the volume suitable for cell ingrowth and cell viability.

In order to keep a suitable compression modulus (tuned via the overall porosity) a decrease in the amount of solvent used is needed in order to be able to use more porogen if the overall porosity should remain constant.

De Groot demonstrated that interconnectivity can also be obtained by mixing the polymer dioxane solution with c-hexane/water and a porogen, thereby introducing liquid-liquid phase separation during cooling [18]. The connection between the micro pores and the macropores could be opened by applying this phase separation process. However, upon implantation it was found that for these types of structures the interconnectivity limits the ingrowth of tissue [20].

The SEM pictures of Fig. 1 show the influence of the amount of solvent on the micropore structure while the overall porosity is kept constant by changing the amount of porogen. The big pores are imprints of the porogen while the small pores are imprints left behind by the crystallized solvent. From all the scaffolds the solvent could be removed, which suggests that a bi-continuous structure is formed. Scaffolds made with a low polymer concentration (picture C) show a high interconnectivity but also with a major amount of ineffective pores while an increase in polymer concentration leads to a decrease in interconnectivity, but also a minor amount of ineffective pores (picture A). Obviously we need the combination of structure A and C. Therefore we needed to improve the interconnectivity of foams like structure A and this could be accomplished

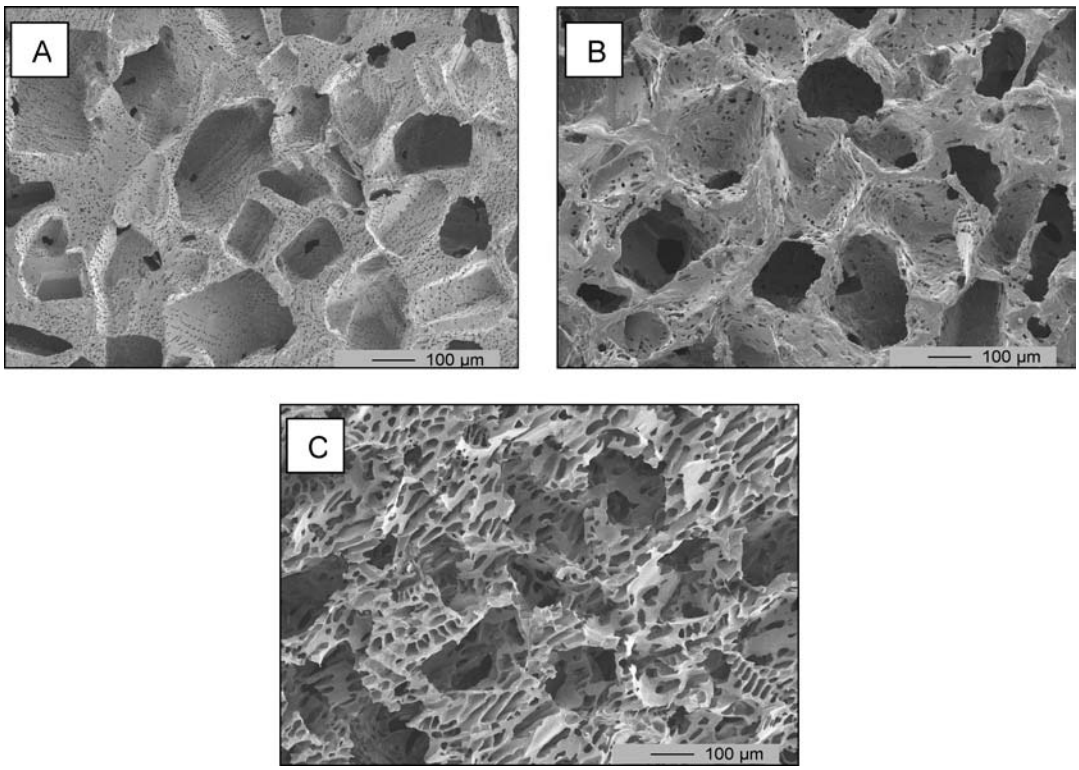

Figure 1 SEM pictures of scaffolds, freeze-dried from dioxane with different polymer concentrations and constant overall porosity (sucrose as porogen and 5% water as non-solvent) A: 35% polymer, porosity: 73.6% B: 30% polymer, porosity: 73.8% C: 20% polymer, porosity 71.6%.

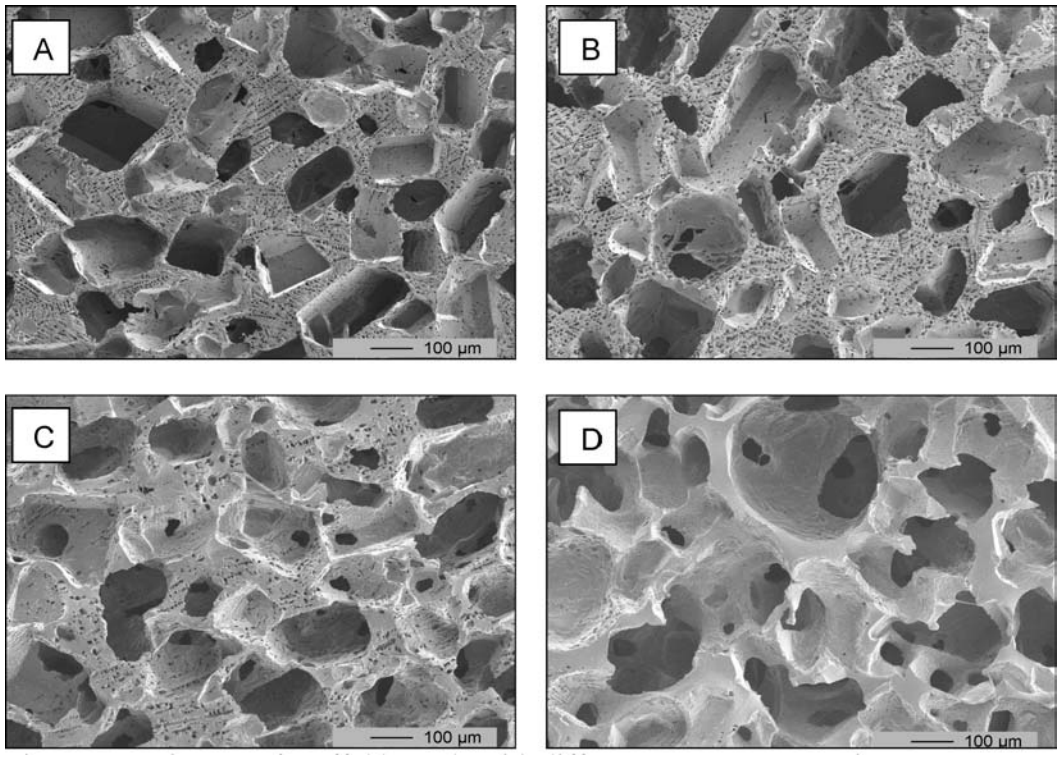

Figure 2 SEM pictures of scaffolds made with different water concentrations at constant polymer concentration (35%) A: 0% water, porosity 72.0% B: 3% water, porosity 76.0% C: 8.7% water, porosity 75.2% D: 10.5% water, porosity 71.8%.

by adding more water inducing liquid-liquid phase separation.

Four scaffolds were made with a polymer concentration of 35 w/w% and 60 w/w% sucrose while the water concentration was varied between 0 and 10.5 w/w% (in relation to the total amount of solvent used). As can be seen in the SEM pictures in Fig. 2 the interconnectivity between the big pores showed a major increase by increasing the amount of water used. The scaffold made in absence of water clearly shows imprints of crystallized solvent. With an increase of the concentration of water these imprints slowly become less visible. The scaffold made with 10.5 w/w% water does not show any effect of the crystallization of the solvent anymore. In this case liquid-liquid phase separation precedes the crystallization of solvent. The quench rate did not lead to significant differences between these foams since this would certainly have lead to differences in structure between the inside and outside of the foams due to the temperature gradient during cooling. The interconnectivity due to liquid-liquid phase separation is in the order of several tens of micrometers. Compared to the interconnectivity due to crystallization of the solvent are these canals much larger and thus more suitable for cell ingrowth.

3.2. Compression modulus

Scaffolds for meniscus regeneration are subjected to compression forces during the initial stages after implantation. Therefore it is necessary to implant a scaffold which is able to cope with these forces as good as possible. This makes the compression properties of the scaffold a key property of the material. A further requirement is that the compressive stiffness of the scaffold should not be too high, as it is thought that compressive forces on the ingrown tissue has a facilitating effect on the differentiation of ingrown tissue into meniscus like tissue [22, 28, 29].

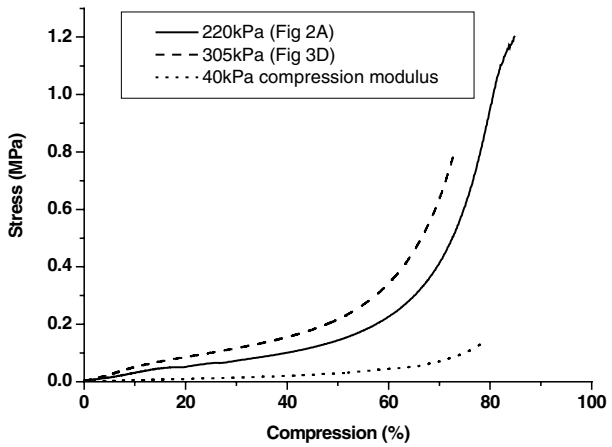

Figure 3 Influence of pore structure (and accompanying compression modulus at 20% compression) on shape of the compression curve.

The two major variables that influence the compression modulus are the porosity and the pore structure. Fig. 3 shows the compression curves of the scaffolds of Fig. 1A, Fig. 2D and of a foam made of the same polymer, but with a very low compression modulus. The modulus value was

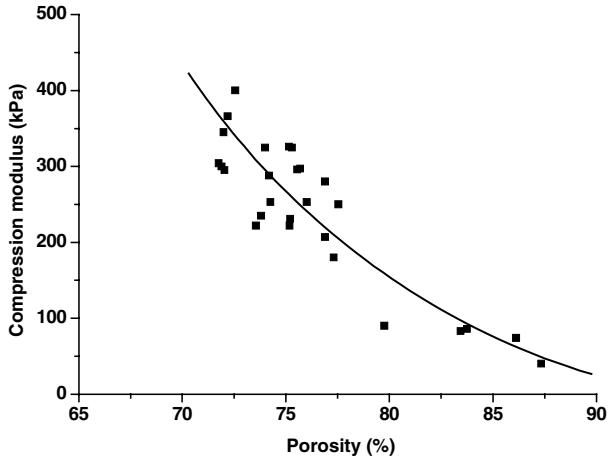

Figure 4 Influence of porosity on the compression modulus at 20% compression.

determined at a compression of 20%. The obtained values are subsequently 220, 300 and 40 kPa. Whether this is representative for the actual mechanical environment as a scaffold *in vivo* remains to be seen. As mentioned before these scaffolds have a completely different porosity and pore structure. Fig. 4 shows the change in compression modulus with variation of porosity without accounting for the pore structure. Even though there is some variation in compression modulus at a constant porosity, a clear trend is visible. A decrease in porosity leads to an increase in compression modulus. The spreading of the measured points at constant porosity might be ascribed to different pore structures of the different scaffolds and this indicates that the pore structure has some, but only a minor influence on the compression modulus compared to the porosity and that the porosity is the factor that determines the compression modulus at 20%. The range of compression moduli that was obtained with different porosities varies between 40 kPa for a porosity of 87% up to a modulus of 400 kPa for a porosity of 73%.

4. Conclusions

Scaffolds were made of the commercially available polyurethane Estane via freeze drying of dioxane (as solvent) and water (as non-solvent) in combination with salt leaching. Without the addition of water as non-solvent the sucrose crystals yielded pores which were poorly interconnected by the imprints left behind by solvent crystals. With the use of water as non-solvent the size of the interconnectivity could be varied, more non-solvent gave a major improvement in the interconnectivity. In the case that enough water was used liquid-liquid phase separation was induced leading to scaffolds with a high degree of interconnectivity. Compared to the materials produced in the past these have the major advantage that the interconnectivity is far better and will limit the ingrowth of tissue less, while also the amount of effective pores increased dramatically. The obtained porosities varied between 72 and 87% with subsequent compression moduli varying between 400 and 40 kPa. The technique was found to be an excellent method to reproducibly prepare highly interconnected and highly porous structures, which might be very suitable as meniscus scaffold.

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