A novel method for fabrication of biodegradable scaffolds with high compression moduli

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It has been previously shown that, when used for meniscal reconstruction, porous copoly(*L*-lactide/ε-caprolactone) implants enhanced healing of meniscal lesions owing to their excellent adhesive properties. However, it appeared that the materials had an insufficient compression modulus to accomplish 100% fibrocartilage formation. In addition, to be used for meniscal prosthesis, the compression modulus of the porous materials should be larger than 150 kPa in order to protect the articular cartilage. A technique was developed to prepare stiff porous materials of a high molecular weight 50/50 copoly(*L*-lactide/ε-caprolactone) suitable for fibrocartilage regeneration in meniscal implants and meniscal prosthesis. Porous microspheres (50–250 μm) were agglutinated in the presence of NaCl crystals (250–300 μm). The microspheres were mixed with solid solvent in order to obtain a homogeneous distribution of solvent over the spheres. By changing the amount of solvent and crystals, the density and the compression modulus could be varied over a range of 0.07 g ml^{-1} to 0.5 g dl^{-1} and 40–1100 kPa, respectively.

1. Introduction

For many biomedical applications, there is a need for porous biodegradable polymeric biomaterials. They are valuable for time-modulated drug delivery systems [1] and as a matrix for the regeneration of tissue [2–12]. Polymer scaffolds have been used for the regeneration of skin [4, 5], blood vessels [6, 7], fibrocartilage [8–10], cartilage [11, 13], and other tissues [14, 15]. Interconnectivity of the pores is a common property that the materials must fulfil in order to allow the ingrowth of tissue, diffusion of nutrients and clearance of wastes. In addition, the polymer should release non-toxic degradation products. The optimal porosity, pore size, porous structure and mechanical properties of the materials depend upon the specific application. In the case of fibrocartilage regeneration in the meniscus experiments have emphasized an optimal pore size of 150–300 μm [8]. Furthermore, the formation of the fibrocartilage was affected by the compression modulus of the implant [12].

Porous polymer scaffolds have been prepared by numerous techniques, including solvent casting/salt leaching [16], thermally induced phase separation (TIPS) [17], solvent evaporation [18] and fibre bonding to form a polymer mesh [19]. Porous materials used for the regeneration of fibrocartilage in the meniscus, were prepared by a combination of TIPS and salt leaching [20]. The TIPS process utilizes freeze-drying in the latest stage and the main requirement of the polymer is solubility. It begins with a single-phase polymer solution at high temperature. Upon cooling, phase separation will occur. Phase separation can be divided into liquid–liquid phase separation, and liquid–solid phase separation which will occur when the solvent freezes. Then, freeze-drying is pursued to remove the solvent from the polymer solution. Sublimation preserves the morphology developed as a result of induced phase separation, leaving behind an open-cell porous polymer with pores smaller than 50 μm. Because pores of 150–300 μm are essential for the formation of fibrocartilage, the salt leaching step was added, in which water-soluble crystals (150–300 μm) such as saccharose were mixed with the polymer solution. After freeze-drying, the crystals were washed out leaving a macroporous structure. The smaller pores arising from sublimation of the solvent served as interconnection between the macropores.

Because strength and resilience of the materials used for meniscal tissue regeneration are very important properties, polyurethanes were first applied. However, the diisocynates in the used polyurethanes may be converted into toxic diamines during degradation. Therefore, in a previous study, a high molecular weight 50/50 copolymer of *L*-lactide and ε-caprolactone was utilized [12]. This copolymer appeared to be an elastomer with mechanical properties comparable to segmented polyurethanes (PU) due to a highly entangled polymer chain and the presence of crystallizable *L*-lactide sequences [21]. Upon degradation the polymer will yield *L*-lactic acid and ω-hydroxy hexanoic acid as degradation products. In addition to releasing non-toxic degradation products, porous copoly(*L*-lactide/ε-caprolactone) implants enhanced healing of meniscal lesions owing to its excellent adhesive properties. As a consequence of the high

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degradation rate, the amount hydrophylic carboxylic and hydroxylic groups formed upon degradation is increased.

It was shown that the formation of fibrocartilage was affected by the modulus of the implant. Copolymer implants with a compression modulus of 40 kPa never showed ingrowth of fibrocartilage, whereas implants with a compression modulus of 100 kPa showed maximal 50%–70% fibrocartilage formation after 10–25 wk implantation. Aliphatic PU implants with a compression modulus of 150 kPa showed maximal 100% fibrocartilage [12]. In order to improve the ingrowth results of copolymer implants, and to take into account the high degradation rate of the copolymer, the compression modulus should be enhanced considerably. Additionally, when used as meniscal prosthesis, a high modulus of the porous material is needed to prevent articular cartilage degeneration. Owing to the high molecular weight of the polymer, increasing of the compression modulus of the materials using the freeze-drying/salt-leaching technique, was not possible.

In this study, alternative production procedures are reported to make porous copolymer materials with high compression moduli. The materials were prepared using an adapted freeze-drying/salt leaching technique and by agglutinate copolymer microspheres in the presence of NaCl crystals of 250–300 μm.

2. Experimental procedure

2.1. Materials

L-lactide (CCA/Purac Biochem, the Netherlands) was purified by recrystallization from dry toluene. ε-Caprolactone (Jansen Chimica, Belgium) was purified by drying over CaH_2 and distillation under a reduced nitrogen atmosphere using a Widmer column. The catalyst, stannous octoate, was used directly from the supplier without further purification.

2.2. Polymerization

Polymerization was carried out in silanized ampoules. Monomers were mixed and an amount of 10^{-5} mol catalyst per mole of monomer was added. Under reduced pressure (1–0.5 Pa), the ampoules were heat sealed. After homogenization at 110 °C, polymerization was continued for 10 d.

2.3. Preparation of porous materials 1

Polymer solution in 1,4-dioxane (5 wt%) was mixed with saccharose crystals, a wetting agent (water, 2-methyl-2-butanol or 2,2-dimethyl-1-propanol) and a non-solvent (c-hexane). The melting point of the solvent/non-solvent mixture decreased to − 11.1 °C. The polymer solution/crystal mixture was cooled to − 20 °C until the solution was frozen. The mixture was freeze-dried under reduced pressure (0.05 mmHg) until the polymer concentration was 10%. Then the mixture was cut into pieces in the frozen state and poured into a mould. After homogenizing the pieces at 45 °C, the mixture was frozen and solvent was removed under reduced pressure and crystals were removed with water, leaving behind the polymer as a foam.

2.4. Microspheres

Microspheres were prepared using the solvent-evaporation method [22]. The polymer was dissolved in dichloromethane to a concentration of 2 wt%. The solution was slowly added to a five-fold excess of a 2 wt% solution of polyvinylalcohol in water under stirring (250 r.p.m.) at 30 °C. The dichloromethane was then evaporated at atmospheric pressure for 7 h. When evaporation was complete, the microspheres were sieved into a fraction > 50 μm and washed with water and ethanol. Then the microspheres were dried in a vacuum stove at 37 °C. The yield under these conditions was 80% and size of the spheres 50–250 μm.

In order to prepare porous microspheres, the polymer solution in dichloromethane was mixed with paraffin oil. The ratio of polymer to paraffin oil was 1:1. After preparation as described above, the paraffin oil was removed by washing with hexane.

2.5. Preparation of porous materials 2

Porous materials were made by agglutination of copolymer microspheres in the presence of NaCl crystals (250–300 μm). Agglutination of the microspheres was achieved by three methods: (a) by sintering at 130 °C under a nitrogen atmosphere for 16 h, using paraffin oil as conduction agent; (b) by mixing the microspheres with a 35/65 wt% 1,4-dioxane/c-hexane mixture at room temperature; (c) by mixing the microspheres with solid 1,4-dioxane. In order to obtain small, solid 1,4-dioxane particles, 1,4-dioxane was mixed with liquid nitrogen and pulverized. Non-solvent and solid were used to obtain homogeneous distribution of the microspheres and solvent.

2.7. Characterization

The effectiveness of paraffin oil removal was determined by 300 MHz ^1H nuclear magnetic resonance. Characterization of the microspheres and pore structure in the materials was performed using an ISI-DS scanning electron microscope.

Stress–strain and compression curves were determined at room temperature using an Instron (4301) tensile tester equipped with a 10 n or 100 n load cell at a crosshead speed of 12 mm min^{-1}.

Tensile strength as function of agglutination time was determined. Rectangular test samples of $5 \times 3 \times 25\,mm^3$ were subjected to tensile testing.

For compression, cylindrical specimens with a diameter of 10 mm and a length of about 8 mm were cut out of the foams by cooling them with liquid nitrogen.

3. Results and discussion

Repair of large meniscal lesions in the avascular part of the meniscus can be achieved by implanting a

porous polymer as a connection between lesion and synovial lining. The implant stimulates the ingrowth of blood vessels and cells into the defect and plays an essential role in the formation of fibrocartilage. Under the right circumstances, the ingrowing fibrous tissue transforms into fibrocartilaginous tissue. Pores of 150–300 μm [8, 23] and the compression modulus of the implant [16] are found to be essential for fibrocartilage formation. When being used as meniscal prosthesis, the compression modulus of the porous material is also important for the protection of the articular cartilage [10].

In a previous study, a porous 50/50 copolymer of *L*-lactide and ε-caprolactone was used for meniscal reconstruction [12]. The materials were prepared using a combination of freeze-drying and salt leaching. The polymer was dissolved in 1,4-dioxane and mixed with saccharose crystals of 200–400 μm and a non-solvent. After freezing of the mixture, sublimation of solvent/non-solvent and washing out the crystals, a porous structure was obtained. Macropores, due to the casting material, were dispersed in a matrix of micropores (<50 μm) as a result of the freeze-drying process.

Owing to the high molecular weight of the polymer, the maximal concentration of the polymer solution to which saccharose crystals could be added homogeneously was only 5% and resulted in a compression modulus of 40 kPa. These materials never showed fibrocartilage formation. To obtain a higher compression modulus, E_f, the density, ρ_f, of the porous material should be increased because, theoretically, they are related by [24, 25]

$$E_f \sim (\rho_f)^2 \quad (1)$$

Therefore, after mixing the polymer solution with the crystals, the polymer concentration was increased by controlled evaporation of the solvent. The maximal compression modulus of porous copolymer using this technique was 100 kPa. This compression modulus, however, was not sufficient to achieve 100% fibrocartilage formation. Furthermore, it was observed that by implanting a porous PU prosthesis with a compression modulus of 150 kPa, degeneration of the articular cartilage, although less severe than after meniscectomy, could not be prevented. Therefore, increasing the compression modulus of porous copolymer materials was necessary.

3.1. Porous materials 1

In order to increase the polymer concentration, a 5 wt% copolymer solution in 1,4-dioxane and c-hexane (90/10) was mixed with 30 wt% saccharose crystals (200–400 μm). After freezing the mixture at -30 °C, freeze-drying of solvent/non-solvent was performed until the percentage of polymer was 10%. Then the mixture was homogenized at 45 °C, frozen at -30 °C and freeze-dried until the solvent and non-solvent were removed. Afterwards the crystals were washed out with water. With this method, materials with compression moduli up to 150 kPa could be made reproducibly. However, this compression modulus has proved to be insufficient to prevent cartilage degeneration in the case of meniscal prosthesis. For the preparation of stiffer materials, another technique is required.

Agglutination of small polymer particles is another option to prepare porous materials. Owing to the high toughness of the polymer at temperatures below the transition temperature, mechanical grinding was impossible. Therefore, microspheres were prepared.

3.2. Microspheres

To prepare microspheres, the solvent evaporation method was used. Biodegradable polyester microspheres made by this technique were first described by Beck *et al.* [22]. The polymer was dissolved in a volatile solvent and suspended in water with an appropriate emulsifying agent. After removing the solvent under reduced pressure and by heating, microspheres can be filtrated. The polymer concentration, stirring rate during emulsifying, concentration and kind of emulsifying agent, and temperature, affect the morphology of microspheres.

In this study, the copolymer was dissolved in dichloromethane. The polymer solution was poured into an agitated aqueous phase. The resulting emulsion was stirred continuously at 30 °C until the solvent evaporation was completed. The microspheres were collected by filtration, washed with water and dried. Fig. 1 shows a scanning electron micrograph of copolymer microspheres.

3.3. Porous materials

Microspheres (50–250 μm) were agglutinated in the presence of NaCl crystal with sizes of 150–300 μm, which were washed out with water afterwards. Crystals were used because the macropores are essential for the formation of fibrocartilage. In order to join together the microspheres, different methods of agglutination were applied. Firstly, the microspheres were sintered at 130 °C using paraffin oil as conduction agent. The porous structure of the material is shown in Fig. 2. Owing to the temperature gradients as a result of the poor conductivity of the polymer, the material

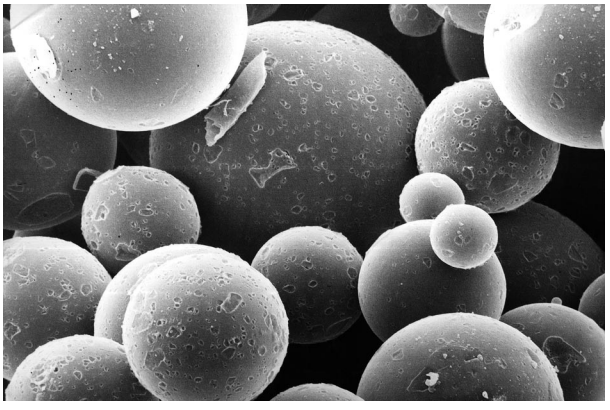


Figure 1 Scanning electron micrograph of copolymer microspheres prepared by the solvent evaporation method.

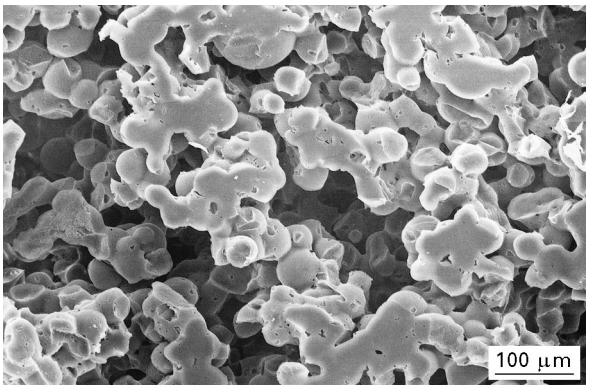


Figure 2 Scanning electron micrograph of copolymer microspheres, which were sintered at 130 °C in the presence of NaCl crystals (250–300 μm) using paraffin oil as a conduction agent.

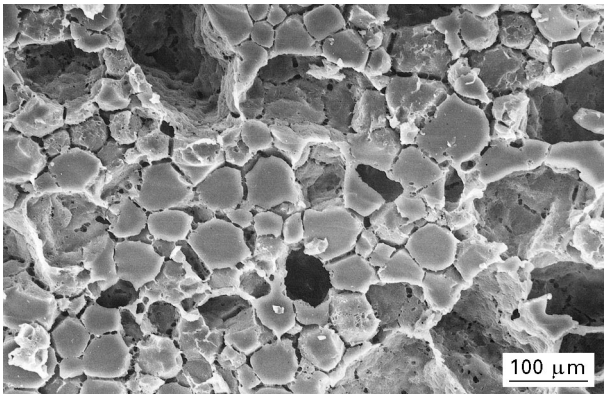
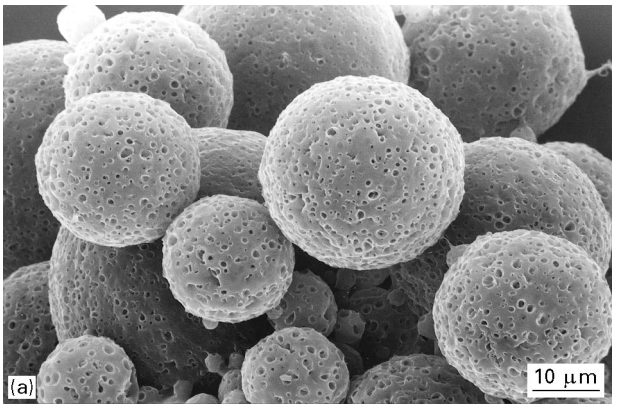


Figure 3 Scanning electron micrograph of porous copolymer made by agglutination microspheres with (35/65 wt %) mixture of 1,4-dioxane and cyclohexane in the presence of NaCl crystals (250–300 μm).



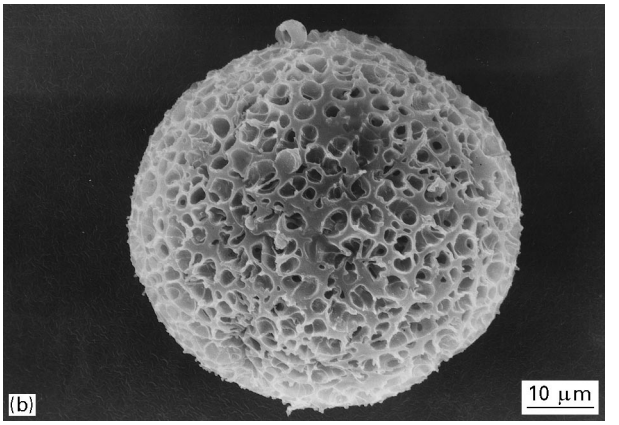


Figure 4 (a) Scanning electron micrograph of porous copolymer microspheres made by the solvent evaporation method combined by adding 50 wt % paraffin oil to the polymer solution: (b) Detail of the microspheres.

was inhomogeneous. At the surface of the material, the microspheres were melted together, contrary to in the core, where disconnected microspheres were visible. At longer sintering time, polymer degradation was observed at the skin, while on the inside loose spheres were still visible.

Secondly, the microspheres were agglutinated using solvent. Agglutination with solvent, 1,4-dioxane, was difficult because homogeneous dispersion of the solvent was impossible owing to the quick adherence of the microspheres. Therefore, the solubility was decreased by adding 65 wt % non-solvent, c-hexane, to the solvent. After agglutination of the microspheres in the presence of NaCl crystals, the solvent/non-solvent mixture was removed by freeze-drying. The porous structure is shown in Fig. 3. The shape of the spheres is still visible owing to the poor solubility of the polymer in the solvent/non-solvent mixture. In addition, the interconnectivity of the macropores as a result of the casting materials, is poor and should be improved to allow quick ingrowth of tissue.

Therefore, porous microspheres were prepared by mixing the polymer solution with 50 wt % paraffin oil, which is a non-solvent for the copolymer. During evaporation of the solvent, liquid–solid phase separation between paraffin and polymer occurred and afterwards the paraffin oil was removed by washing the microspheres with *n*-hexane. Fig. 4a shows a scanning electron micrograph of porous microspheres with a porosity of 45% and Fig. 4b shows a scanning electron micrograph of one porous microsphere.

Decreasing the solubility of the copolymer of the solvent was necessary to distribute homogeneously the solvent/non-solvent over the microspheres before agglutination. The result, as shown in Fig. 3, was however, not satisfactory. Therefore, the solvent quality was temporarily decreased by freezing the solvent. In order to do so, 1,4-dioxane was frozen with liquid nitrogen and pulverized, after which it was mixed with porous copolymer microspheres and NaCl crystals. The spheres were allowed to agglutinate at room temperature. Afterwards the solvent was removed by freeze-drying and the crystals were washed out with water. The resulting porous structure is shown in Fig. 5. During agglutination, a skin formed around the porous microsphere which resulted in a poor interconnected pore structure. A better interconnected structure was obtained when the paraffin oil was removed after agglutination, freeze-drying and washing out the crystals. The resulting porous structure is shown in Fig. 6. The spheres are completely agglutinated to each other and are no longer visible. The macropores due to the casting material, are dispersed in a matrix of micropores smaller than 50 μm. This porous structure is comparable to previously used PU implants.

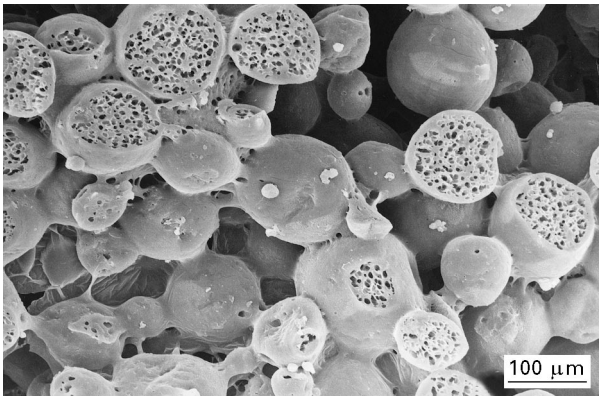


Figure 5 Scanning electron micrograph of porous copolymer made by mixing porous microspheres with solid solvent (1,4-dioxane) and NaCl crystals (250–300 μm). After agglutination of the spheres at room temperature, dioxane was removed by freeze-drying and crystals were washed out with water.

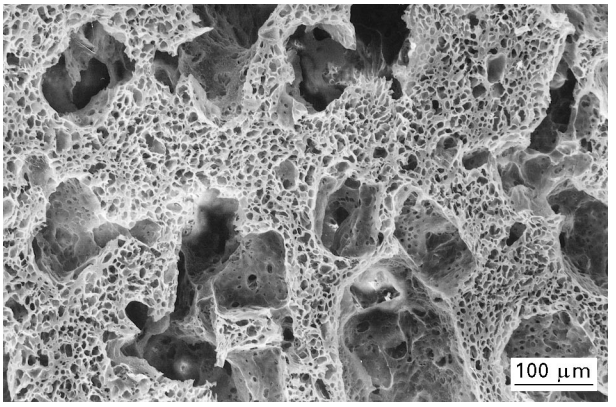


Figure 6 Scanning electron micrograph of porous copolymer. The material was prepared using porous microspheres in which the paraffin oil was still present. Paraffin oil was removed after agglutination, freeze-drying, and washing out of the crystals.

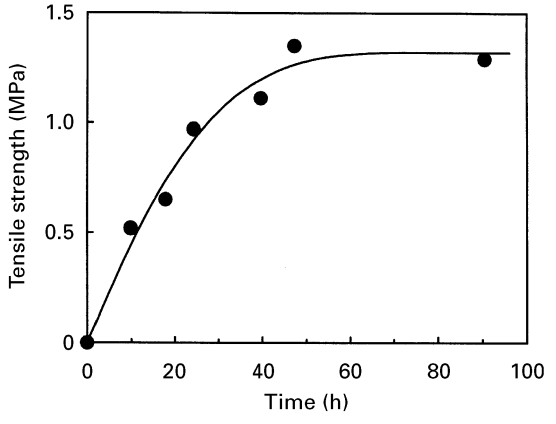


Figure 7 The tensile strength of porous copolymer, made by agglutination of microspheres, as function of agglutination time.

In order to obtain materials with a maximal strength, the tensile strength of the porous materials was measured as a function of agglutination time. From Fig. 7, determined for microspheres with a porosity of 76%, it can be concluded that the minimal required time is 2 d.

By varying the crystal concentration, the compression modulus could be varied. As was mentioned before, the Young's modulus of open-cell porous materials and the density are theoretically related by [24, 25]

$$E_f/E_s = (\rho_f/\rho_s)^2 \quad (2)$$

where E_f and E_s are the Young's moduli of porous and solid materials, respectively, and ρ_f and ρ_s are the respective densities. The compressive stress–strain curves of the porous copolymer exhibit linear elasticity at low stresses. It was followed by a collapse plateau, owing to buckling of the walls, which is truncated by a regime of densification in which the stress rises steeply. As the porous materials used for meniscal prosthesis and meniscal reconstruction are highly stressed, the modulus at this collapse plateau, which is positioned at 10%–25% compression, is very important. Therefore, the compression modulus at 20% compression as a function of density is determined. Fig. 8 shows the compression modulus at 20% compression as a function of the relative density of the porous materials. The logarithm of the compression modulus at 20% compression is plotted against the logarithm of the relative density in Fig. 9, and falls on a linear curve with a slope of 1.8, approaching the theoretical value of 2. Although the relationship between the collapse stress and the density is more complicated because the densification has to be taken

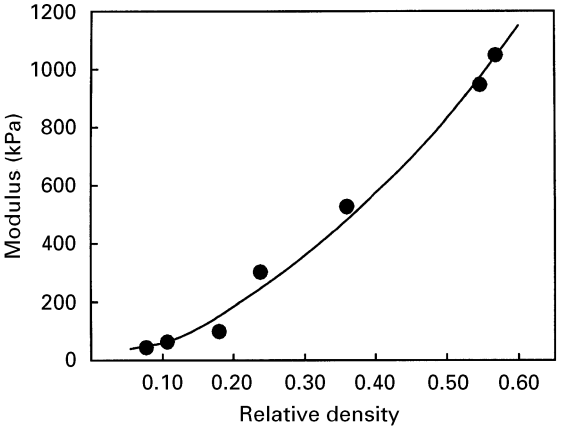


Figure 8 Relationship between the compression modulus at 20% compression and relative density of porous copolymer.

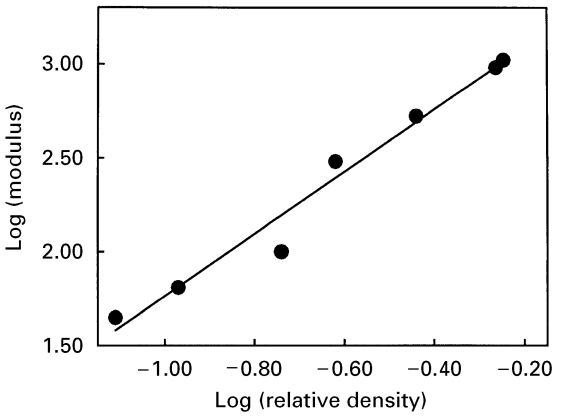


Figure 9 Relationship between the logarithm of the compression at 20% compression and the logarithm of the relative density.

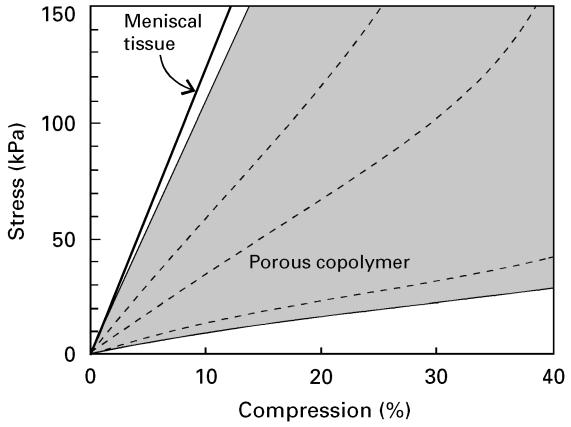


Figure 10 The range (grey area) in which the compression behaviour of materials can be varied compared to (——) the compression behaviour of dog meniscal tissue.

into account [24], the data of the porous copolymer are fitted approximately by the simple Equation 2. Materials over a wide range of densities and compression moduli, from $0.07\,g\,m^{-1}$ to $0.5\,g\,dl^{-1}$ and 40–1100 kPa, respectively, could be made reproducibly. The range in which the compression behaviour of materials could be varied and the compression behaviour of dog meniscal tissue is shown in Fig. 10. Thus, using this technique, porous materials with compression moduli approaching meniscal tissue could be made.

4. Conclusions

Agglutination of porous microspheres in the presence of NaCl crystals has proved to be an excellent method to prepare reproducibly stiff porous materials of a high molecular weight copolymer of *L*-lactide and ε-caprolactone. The resulting porous structures are comparable to the structures of previously implanted porous PUs. By mixing microspheres with solid 1,4-dioxane, premature adhesion could be prevented and the solvent could be homogeneously distributed over the microspheres. With this technique, porous copolymer materials could be prepared reproducibly over a wide range of densities and moduli. The fact that the copolymer will release non-toxic degradation products and possesses excellent adhesive properties, makes these materials suitable for meniscal reconstruction and meniscal prosthesis.

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