Water as foaming agent for open cell polyurethane structures

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The problem of moisture in polymer processing is known to any polymer engineer, as air bubbles may be formed. Hence granulates are generally dried prior to manufacturing. This study tried to develop a novel processing methods for scaffolds with controlled moisture content in thermoplastic polyurethane. The common foaming agents for polyurethane are organic solvents, whose residues remaining in the scaffold may be harmful to adherent cells, protein growth factors or nearby tissues.

Water was used as a foaming agent and NaCl was used as porogens to achieve an open-cell structure. The polyether–polyurethane samples were processed in a heated press, and achieved a porosity of 64%. The pore size ranged between 50 and 500 μm. Human fibroblasts adhered and proliferate in the scaffold.

A non-toxic production process was developed to manufacture a porous structure with a thermoplastic polyether–polyurethane. The process enables a mass-production of samples with adjustable pore size and porosity.

In contrast to an existing method (solvent casting), the processing of the samples was not limited by its thickness. The process parameters, which attribute mostly to the pore building, were filling volume, temperature, NaCl-concentration and water-uptake rate.

Introduction

Porous polymers play a key role in the development of tissue engineering as they serve as scaffolds for cellular attachment and tissue development [1]. Current approaches for fabricating porous biodegradable polymer structures include solvent casting, particulate leaching, phase separation, emulsion freeze drying, carbon dioxide expansion, and combinations of these. All the above mentioned techniques use organic solvents which may be left in the scaffold and might cause inflammatory reactions [2] and hence no ideal scaffold processing method is yet known.

Certain polymers have to be dried prior to processing in order to remove air bubbles in the product. A study has been done to investigate whether this problem can be reversed into a helpful tool for foaming of polymers. The most common foaming agents for polyurethane are solvents [3]. By using moisture one would have a novel non-toxic foaming method.

Material and methods

NaCl (VWR, Darmstadt, Germany) were grinded in a planetary mono mill (Pulverisette 6, Fritszch, Idar-Oberstein, Germany) and sieved (Sieve 100 and 200 μm, Retsch, Haan, Germany) in a sieve tower (AS 200 Retsch, Haan, Germany) to obtain the desired particle size of 100–200 μm. Thermoplastic polyether–polyurethane (TPU) (Texin, Bayer AG, Leverkusen, Germany) was compounded with these particles in a double-screw extruder (ZK 25 × 36 D, Dr Collin, Ebersberg, Germany), where granulates of 3 mm with the a well-distributed salt particles were obtained.

The granulates were placed in a controlled atmosphere (50 rel %) in order to adsorb a defined moisture content. A moisture analyser (MA 100, Sartorius, Ismaning, Germany) was used to monitor this uptake.

Later these granulates were filled into a Teflon mould with 10 cylindrical chambers (Diameter 10 mm, Height 5 mm), and heated up to 230 °C in a heated press (300 P, Dr Collin, Ebersberg, Germany) where the moisture rapidly expanded and created macropores. These closed pores were opened by leaching out the salt particles.

Mercury intrusion porosimetry (AutoPore VI 9500, Micromeritics GmbH, Mönchengladbach, Germany) was used to determine pore size distribution and porosity of the samples. Scanning electron microscopy (S3500-N, Hitachi, Japan) was used for the observation of the internal pore morphology of the samples. The samples were sliced by a scalpel and then coated with gold by using a sputter-coater (SCD 005, BAL-TEC AG, Balzers, Lichtenstein).

Mechanical tests were performed on an universal tensile strength machine (Zwicki, Zwick Roell, Ulm,

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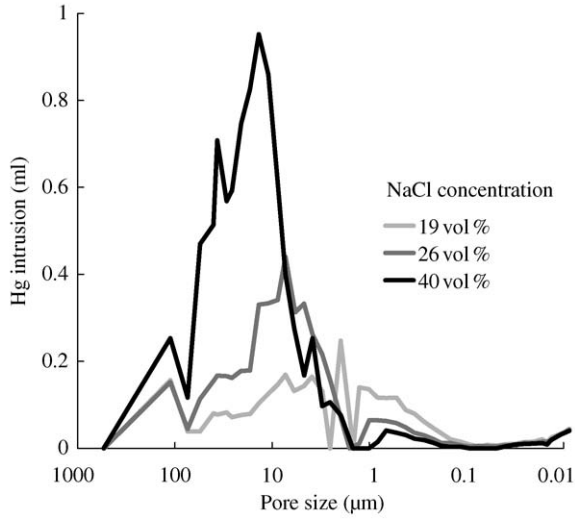

Figure 1 Pore distribution as a function of NaCl-concentrations.

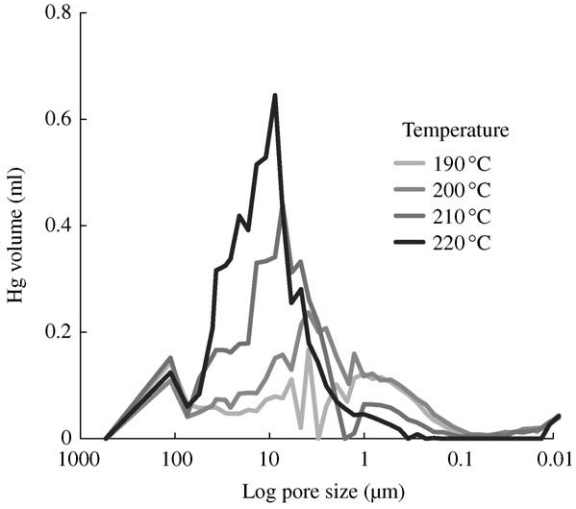

Figure 2 Mercury intrusion measurements of the samples as a function of processing temperatures.

Germany) with parameters described in the German industrial norm DIN 1798.

Chemical changes in the polyether–polyurethane were monitored by an annotated total reflexion (ATR) Fourier transform–infrared (FT–IR) spectroscopy (Spectrum One, Perkin Elmer, Rogau-Juegesheim, Germany).

For an initial cell attachment study, human fibroblast (Detroit 551, ATCC, Manassas, VA, USA) were seeded onto the scaffold. Proliferation was evaluated by a colorimetric assay (WST-1, Roche, Penzberg, Germany). The 96-well-plates with TPU scaffolds were filled with 0.4 ml Dulbecco's Modified Eagle Medium DMEM (Biochrom AG, Berlin, Germany) and cell density of 10 200 cells/well. These were then read on an ELISA Reader (Sunrise, Tecan, Crailsheim, Germany) using a wavelength of 450 nm and a reference wavelength of 620 nm.

Results and discussion

The parameters which greatly contributed to the porous structure were moisture content, processing temperature and NaCl content. Less effect were heating and cooling rates.

Parameters were found to play a different role in pore size and porosity. By increasing the NaCl content and processing temperature, one clearly could observe a rapid change in porosity. The mercury intrusion data (Figs. 1 and 2) show that for these two parameter variations, only the area under the graph is changing, whereas the peaks are almost constant. This result shows that processing temperature and salt concentration can be used to adjust the porosity.

An increase in temperature makes the polymer more viscous, and the pores from the rapid expansion of the moisture can be fully developed. At lower temperature the pores were deformed. This phenomenon was observed in SEM (Fig. 3), where the left image clearly shows underdeveloped pores, and the right image displays fully spherical ones.

To control the pore diameter, the moisture content taken up by the polymer played a major role. From Fig. 4 it is evident that the increased moisture content did not greatly change the porosity as the cross section areas under the graphs are almost constant. The peaks however are shifted to the left by increasing moisture content, which indicates an increase in pore size.

By optimising the processing parameters, an ideal method was found. These parameters were: 70% mould

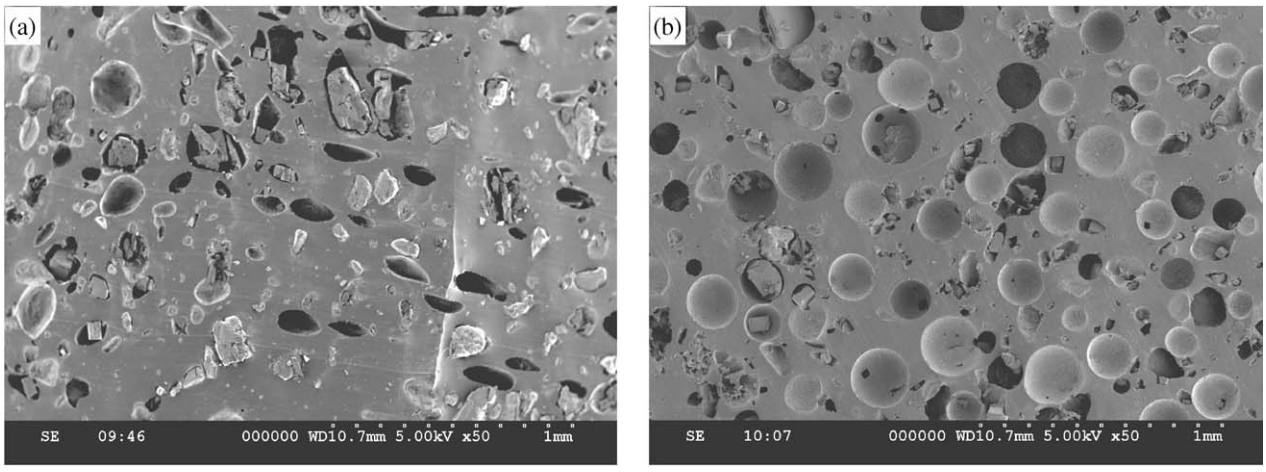

Figure 3 Cross section of a sample produced at a temperature of 190 °C shows small underdeveloped vapour pores and entrapped NaCl particles (a), Cross section of a sample produced at a temperature of 220 °C shows large vapour pores with a spherical shape (b).

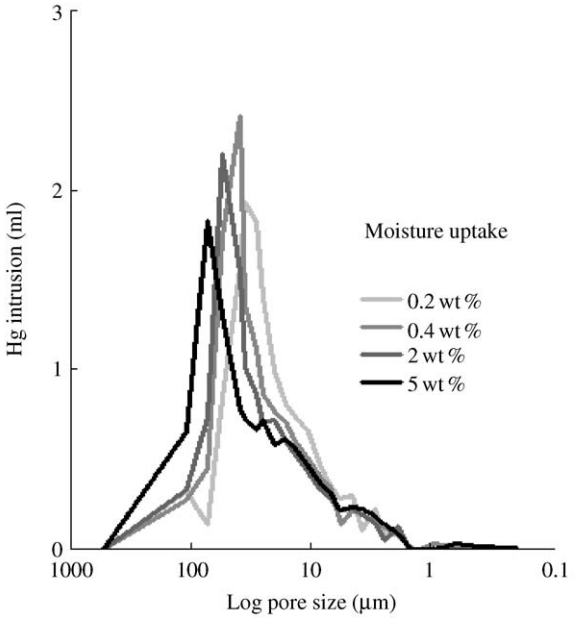

Figure 4 Mercury intrusion measurements of the samples as a function of different moisture-uptake rates.

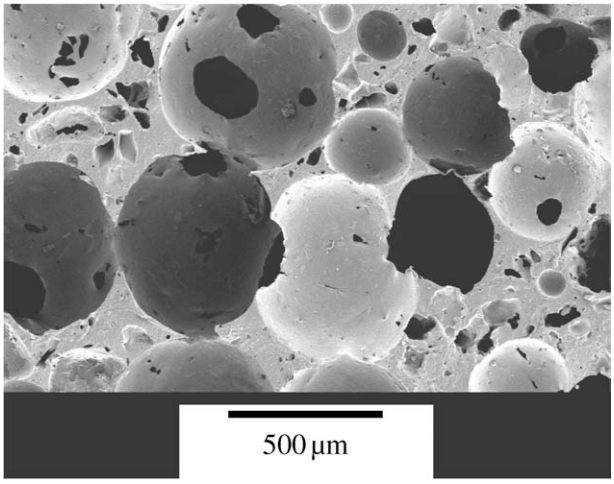

Figure 5 SEM picture of TPU scaffold made by optimum processing parameters.

TABLE I Tensile strength test after DIN 1798

Young's Modulus	10.53 ± 3.83 MPa
Tensile strength	2.03 ± 0.98 MPa
Elongation at break	$56.59 \pm 16.13\%$
Tension at 0.5% elongation	0.12 ± 0.02 MPa
Tension at 5.9% elongation	0.61 ± 0.04 MPa
n	16

filling, NaCl content of 40 vol %, processing temperature of 230 °C, processing time 540 s and moisture uptake of 5 wt %. With these parameters scaffolds with a porosity of 65% and pore size between 50 and 500 μm (see Fig. 5) could be produced. By using these parameters, dogbone-shaped (dimension described in DIN 1798) were made and used for mechanical testing ($n = 16$) and gave tensile strength of 2.03 ± 0.98 MPa and Young's Modulus of 10.53 ± 3.83 MPa. Further mechanical result is listed in Table I.

An ATR FT–IR analysis was performed to test whether the chemical structure of the TPU had been changed by the fairly high processing temperature and presence of moisture during processing. Fig. 6 shows this comparison and it is evident that only minor changes have occurred. The only significant differences were at 1525 cm^{-1} for the d(N–H) + v(C–N) bonds, at 1218 cm^{-1} for the $w(CH_2)$ bond and at 1070 cm^{-1} for the v(C–O–C) bond with differences in absorption of 3.2%, 3.7% and 2.17% respectively.

The WST-1 array test showed no significant difference in proliferation compared to the positive control (Fig. 7). The human fibroblasts adhered to the scaffold (Fig. 8).

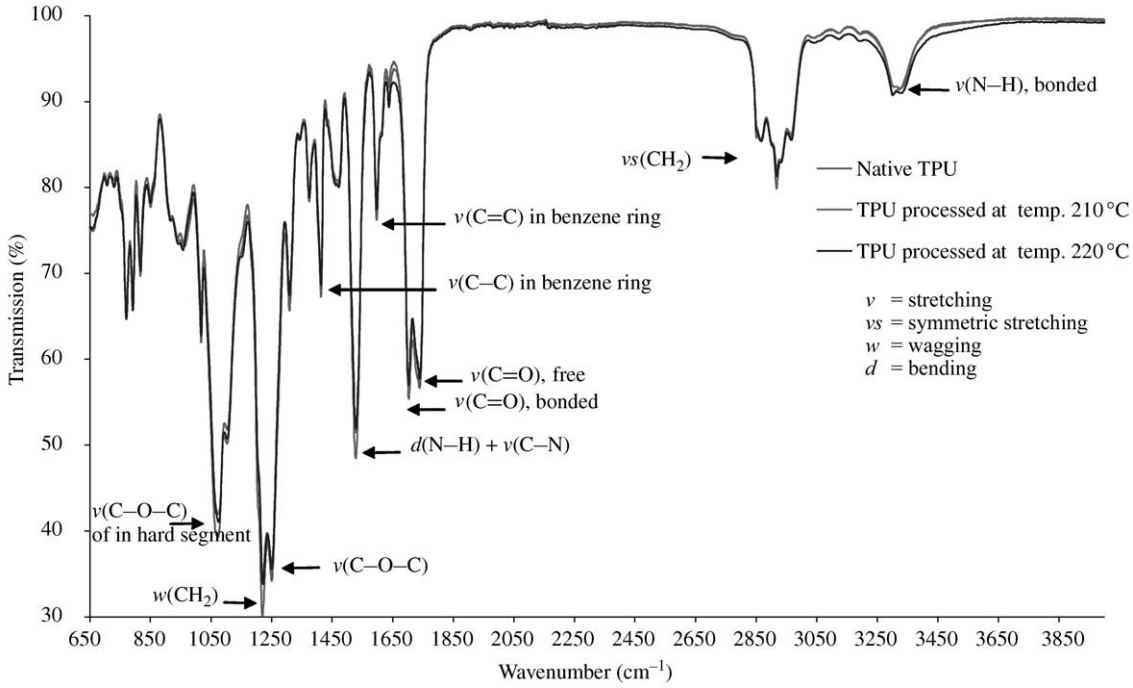

Figure 6 ATR FT-IR analysis of the scaffold processed at 210 °C and 220 °C compared with native, unprocessed TPU.

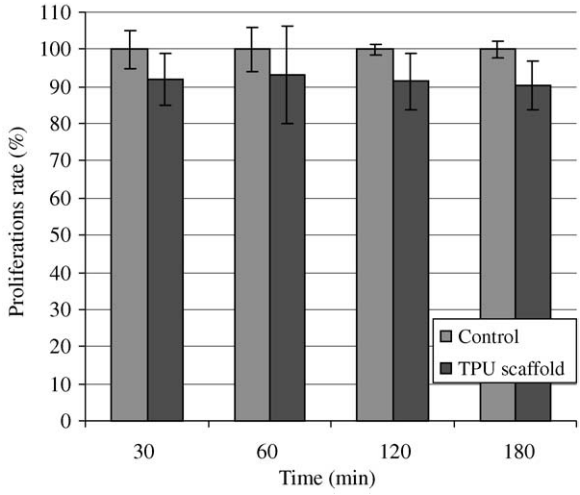

Figure 7 WST-Proliferation test of human fibroblast on TPU scaffold.

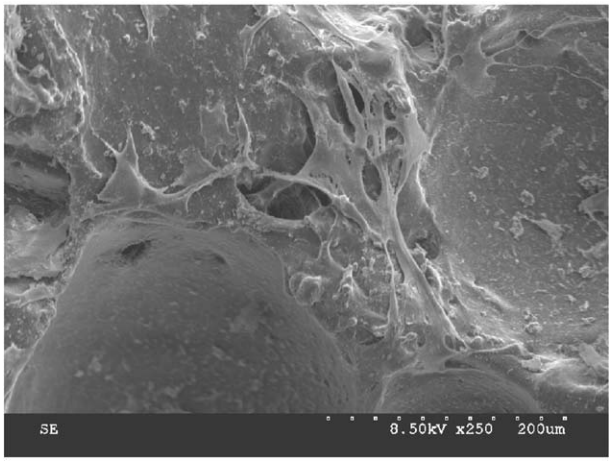

Figure 8 Human fibroblasts attached in the TPU scaffold.

Conclusion

The pore size is adjustable on the amount of water absorbed, porosity was found to be heavily dependent on processing temperature, mould filling volume and the NaCl content. Cooling and heating rate was independent upon the porous structure. The NaCl content (40 vol %) and moisture content (5 wt %) was limited by mechanical strength, and above these limits, the scaffold could poorly be handled.

It seems like a new scaffold processing method without any use of toxic organic solvents, has been established, with the needed scaffold structure (interconnective pores, adjustable pores size and porosity).

One has to make clear that TPU is not suitable for high mechanical loads (tensile strength of 2.03 ± 0.98 MPa and Young's Modulus of 10.53 ± 383 MPa) and would be more appropriate for soft tissue regeneration. The polymer discs had a mean pore size of 100 µm and porosity $> 65\%$, and were interconnective (see Fig. 4). The largest pore sizes were 500 µm. The processing temperature showed little effect on the chemical composition from ATR FT–IR analysis.

Future studies will involve processing of porous structure with this method on an injection moulding machine (C2 520, Krauss Maffei, München, Germany) to try to improve the mechanical properties. The porosity is expected to increase.

References

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