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Manufacturing of porous polymer films as a basis for the novel cardiovascular implant devices

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Abstract:
For applications in the cardiovascular field, the production of tailored biomaterials such as those required for insulations or patches is a key technology. In this work we were able to manufacture thermoplastic silicone polycarbonate elastomer scaffolds with a porous external surface. The samples were produced using the dip coating process. Pores were created using the salt leaching technique. The complete removal of the salt was verified by Raman microscopy. The absence of solvents was demonstrated by biocompatibility tests using an endothelial cell line. The potential functionality of the system was thus demonstrated. The next step will focus on promoting the adhesion of suitable cells for specific applications.

Keywords: Salt leaching, porosity, polymer film, biocompatibility, Raman spectroscopy.

1 Introduction

Polyurethane and its derivatives like thermoplastic silicone polycarbonate elastomer (PCU-co-Si [1, 2]) are materials that are becoming increasingly popular for permanent implant devices. Electrode insulations, for example [3], are made of it, but it is also of interest for other permanent implant structures or coatings, for example in the cardiovascular field [4]. However, it was shown that polyurethanes can degrade *in vivo* over time due to oxidative stress [5]. Manufacturers of polymers classified as biomedical grade are therefore working on improving material stability, for example by copolymerizing them with silicone [2]. In addition to stability, it is essential that the material is sterilizable and processable, and that it meets the required mechanical properties depending on the intended use. The material used here is biocompatible if it is processed appropriately, e.g. by completely removing solvent residues via vacuum [1, 6]. However, this does not automatically mean that certain cell types will grow on the surface of the material. In order to make PCU-co-Si more attractive for specific cell types, pores with a pore size of several hundred microns should be inserted into the biomaterial [7, 8]. In addition to various other ways [9], this is possible by adding salt during material processing, which is removed again in a separate step after the material has cured.

2 Materials and methods

2.1 Film preparation

To prepare the polymer solution, 12 g of PCU-co-Si was first dissolved in 400 mL of chloroform. In a dip coating process, eight layers of the solution were then deposited on an immersion core. Subsequently, 20 g of manually crushed NaCl was added to 100 mL of polymer solution and the salt-containing polymer layer was then applied to the base coat in further eight dipping processes. Before each dipping, the salt was slurried by stirring. After finishing the dip coating, the samples were left to rest for 24 hours at room temperature. The films were then cut from the immersion cores and subsequently washed in a water bath on the shaker at 37 °C for seven days, with the distilled water being changed daily. The films then were dried for one week at 40 °C in a vacuum drying oven.

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2.2 Cell culture and cytotoxicity testing

All media components were purchased from PAN Biotech (Germany). Human endothelial cells EA.hy926 (ATCC) were maintained in Dulbecco's Modified Eagle's Medium/10% fetal calf serum (FCS))/antibiotics (pen/strep) at 37°C, 5% CO_2 under humidified atmosphere. For cytotoxicity testing 5.000 cells per well were used. Material extracts were prepared according to DIN EN ISO 10993 and used for cell treatment, followed by viability testing using the CellQuantiBlue assay (BioAssaySystems, Hayward, USA) according to the manufacturer's instructions. Briefly, CellQuantiBlue reagent was added to each well at a final concentration of 10%. The resulting resorufin fluorescence was measured after 2 hours at an emission wavelength of 590 nm with an excitation wavelength of 544 nm using a microplate reader (FLUOstar OPTIMA, BMG Labtech, Offenburg, Germany). Data was normalized to cells treated with cell culture medium without prior material contact.

2.3 Visualization by means of Raman spectroscopy

2.3.1 Overview image

Raman measurements were performed using a confocal Raman microscope Alpha300R (WITec GmbH, Ulm, Germany). Overview images of all samples with an area of 3.000 µm x 3.000 µm were recorded by means of a Zeiss EC "Epiplan-Neofluar" 10x objective (NA 0.25). This was achieved by stitching 36 individual images.

2.3.2 Analysis via area scan measurement

Raman measurements were performed within an area of 500 µm x 500 µm ("area scan") with 100 x 100 measuring points. An integration time of 1 s per measuring point was used. A Raman spectrum was recorded at each measurement point. The visualization of the measurement points was conducted via false colors. Areas in which NaCl was predominantly present (based on the Raman spectra) were colored red. Corresponding areas for PCU-co-Si were colored blue. Regions with low Raman intensity were colored black.

An output power of a Nd:YAG 532 nm laser of 10 mW was used. Measurements were also performed using a Zeiss EC "Epiplan-Neofluar" 10x objective (NA 0.25) (Carl Zeiss AG, Oberkochen, Germany).

3 Results and discussion

The production of a cast film from a polymer/solvent mixture results in a compact material without small cavities or large holes. In contrast, the salt leaching technique integrated into the dipping process results in a heterogeneous 3-dimensional surface (Figures 2A – 2C). The figures also include visualizations of salt and polymer localization (based on the distribution of the respective spectra (Fig. 1) in the area scan).

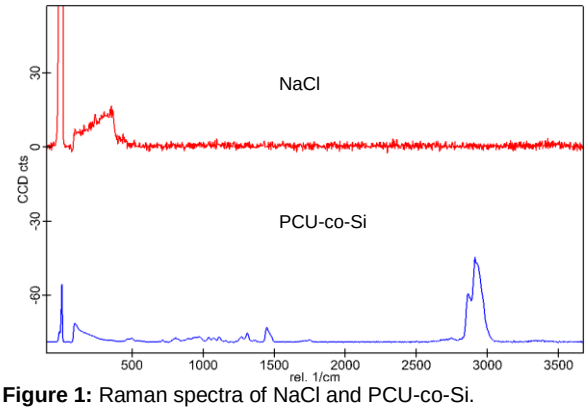


Figure 1: Raman spectra of NaCl and PCU-co-Si.

The absence of a uniform blue color in the entire image section with the overlaid area scan of the Raman microscopy (Fig. 2A) is due to the fact that the polymer film was not exactly in the measuring plane of the Raman microscope due to its curvature. In other words, the intensity of the blue tone is also related to the focusing of the sample. However, washed-out areas can also be seen. After sixteen hours they were partly still occupied with NaCl (Fig. 2B). After five days washing more of the pores and no more salt were found (Fig. 2C). Resulting pore sizes of 100-300 µm were found.

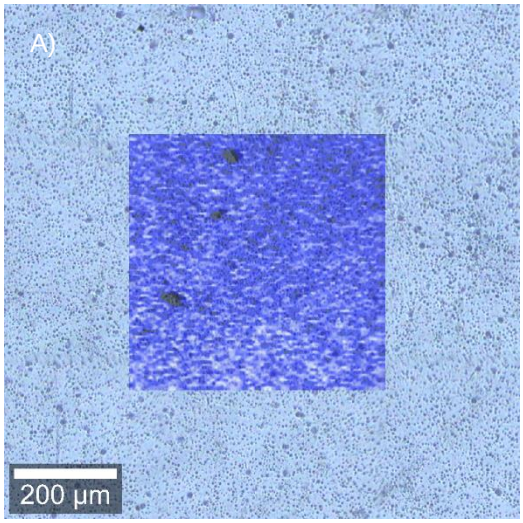
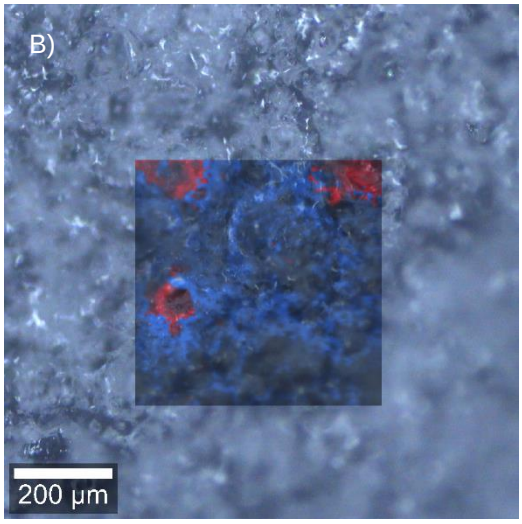


Figure 2: A) Raman visualisation of PCU-co-Si (blue) within a cast film (manufactured without salt leaching technique).



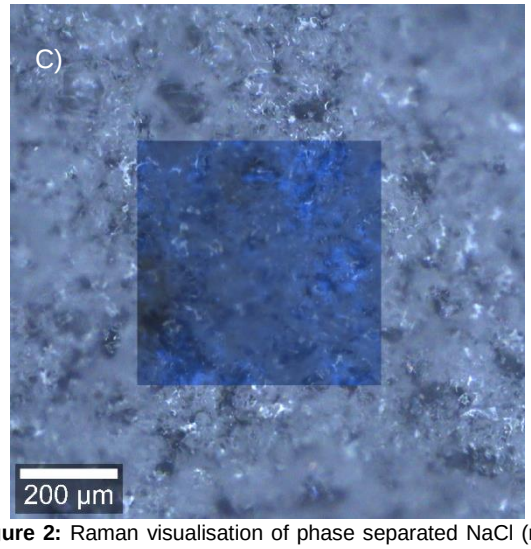


Figure 2: Raman visualisation of phase separated NaCl (red) and PCU-co-Si (blue) within the dip-coated polymer film surface after 16 hours (B) and 120 hours (C) of water washing.

In the next step, the biomaterial films were subjected to cytotoxicity testing according to DIN EN ISO 10993-5. Since endothelial cell line blood vessels and come into direct contact with cardiovascular implants, the human endothelial cell line EA.hy926 was used for this purpose. The metabolic activity of cells treated with the extract of polymer without salt additive as well as salt leached/washed PUR-co-Si films was above 70% (Figure 3). Thus, this materials can be regarded as non-cytotoxic according to DIN EN ISO 10993-5. Lower cell viability in samples treated with salt-free PUR-co-Si extracts suggest that the processing method for this material can even be improved further.

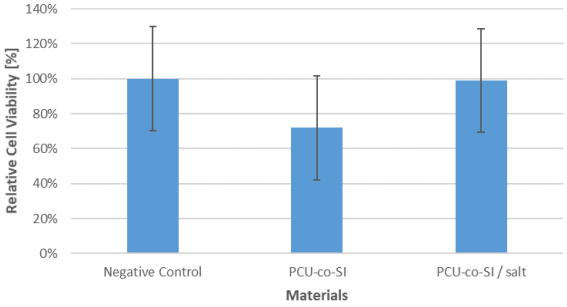


Figure 3: Cytotoxicity testing of PCU-co-Si material extracts (*n* = 3, ± SD, negative control = 100%).

4 Conclusion

It was shown that porous PCU-co-Si films for cardiovascular applications could be produced using NaCl leaching technique. The complete removal of sodium chloride by the applied protocol was demonstrated via Raman microscopy. The biocompatibility of the material was proven by the cytotoxicity testing. The final pore size can be adapted to the application by varying the salt content. However, this is intended to be done in one of the next steps. Scanning electron microscopy is planned for a better visualization of the pores.

Author Statement

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