Valeria Khaimov*, Daniela Koper, Bradley Merrywether, Niels Grabow, Klaus-Peter Schmitz and Stefan Siewert

Polyurethan-based scaffolds for cardiovascular implant devices

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Abstract: Polymeric per- and polyfluoroalkyl substances (PFAS), such as polytetrafluoroethylene (PTFE) and its expanded form (ePTFE), are widely used due to their exceptional chemical resistance and biocompatibility, particularly in medical devices. However, environmental and health concerns linked to PFAS persistence and contamination have prompted the European Union to propose restrictions on their non-essential uses. This has raised challenges for industries dependent on PFAS-containing materials, especially in biomedical applications. In response, this study explores polyurethane (PU) as a potential alternative to ePTFE. Three PU variants were evaluated for their mechanical and biological properties, aiming to identify suitable substitutes that maintain the performance required for critical medical applications.

Keywords: endothelialization, polyurethanes.

1 Introduction

Polymeric per- and polyfluoroalkyl substances (PFAS) are persistent substances that do not degrade, but accumulate over time and are therefore considered harmful to the environment. Polytetrafluoroethylene (PTFE), also known as Teflon, is the best-known member of polymeric per- and polyfluoroalkyl substances (PFAS). Excellent biocompatibility, chemical resistance and high mechanical strength are just some of the valued properties of PTFE that make it almost indispensable in everyday life. Expended PTFE (ePTFE) is a porous expanded form of PTFE widely used in medical devices i.e. stents due to its excellent biocompatibility and inertness [1]. There is no indication that PFAS are released from medical devices and could cause adverse effects. However, health concerns for humans arise as PFAS resulting from the manufacturing process could reach drinking water and food. PFAS exposure to humans was associated with cancer, immune system impairements, developmental effects, etc. Thus, the EU aims to ban non-essential uses of all PFAS as a preventive measure [2]. To this end five EU representatives (Denmark, Germany, the Netherlands, Norway and Sweden) have prepared a proposal aiming to restrict around 10000 PFAS and submitted it to ECHA (European Chemicals Agency) under the REACH (Registration, Evaluation, Authorisation, and Restriction) regulation, which manages chemical risks for human health and environment. This proposal is being currently evaluated and is expected to be published by the end of 2025 [3].

At the same time, industry representatives that rely on ePTFE, such as medical device manufactures have expressed concern about the lack of substitutes and the potential impact on critical applications. Thus, there is a strong need for alternatives to ePTFE with similar mechanical and biological properties.

Polyurethane (PU) is a class of polymers with soft and hard segments within the polymer architecture being responsible for their versatile mechanical features. PUs are used in a wide range of biomedical applications not only due to their excellent mechanical properties, processability and durability, but also due to a very good biocompatibility [4]. In this study, we wanted to characterize three important members of the PU family, polyether urethane (PEU), thermoplastic urethane (TPU) and polycarbonate urethane (PCU), with regard to their mechanical and biological properties.

***Corresponding author: Valeria Khaimov:** Institute for ImplantatTechnology and Biomaterials e.V., Friedrich-Barnewitz-Strasse 4, 18119 Rostock-Warnemünde, Germany, e-mail: valeria.khaimov@iib-ev.de
Daniela Koper, Bradley Merrywether, Klaus-Peter Schmitz, Stefan Siewert: ImplantatTechnology and Biomaterials e.V., 18119 Rostock-Warnemünde, Germany
Niels Grabow: Institute for Biomedical Engineering, Rostock University Medical Center, Rostock-Warnemünde, Germany

2 Materials and methods

2.1 Scaffold fabrication

Homogeneous polymer solutions were obtained by dissolving polyether urethane (PEU) granulate (Biomerics, USA) in tetrahydrofuran, and thermoplastic polyurethane (TPU, Lubrizol, USA) and polycarbonate urethane (PCU) granulate (AdvanSource, USA) in chloroform. The polymer solution was poured into a glass petri dish and left to evaporate until a film of ~100 µm was formed. Polymer films were washed twice using methanol and water. For cell-based investigations circular punches of 6.5 mm were made.

2.2 Tensile measurements

Tensile measurements were performed using a ZwickRoell Z0.5/TN universal testing machine (Zwick GmbH & Co. KG, Ulm, Germany). All tests were performed in accordance with DIN EN ISO 527. Therefore, the specimens for the uniaxial tensile tests were examined using the specimen geometry specified in standard test specimen 1BB.

2.3 Cell culture

All media components were purchased from PAN Biotech (Germany). Human plasma was from Affinity Biologicals Inc (Canada). Human endothelial cells EA.hy926 (ATCC, USA) 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, $5*10^3$ cells/well were seeded in a 96 half area microwell plate (Greiner-Bio-One, Austria) 24 h prior to treatment with material extracts. Material extracts were prepared according to DIN EN ISO 10993-12 and used for cell treatment. 24 h later resazurin-based viability assay (CellQuantiBlue, BioAssaySystems, USA) was performed using a FLUOstar Omega platereader (BMG Labtech, Germany) at ex540/em590. Data was normalized to cells treated with medium that had no contact to polymers during the extraction.

2.4 Microscopy analysis

Blank polymer scaffolds were directly subjected to scanning electron microscopy (SEM) analysis on a Quanta FEG 250 (FEI Company, Germany).

For the microscopy of polymer materials seeded with cells, cut outs of scaffolds were fixed in a 96-well-microtiter plate with teflon rings seeded with $5*10^3$ cells/well for 24 h. Cells were rinsed with phosphate buffered saline (PBS) and fixed in 3,7% formaldehyde (Sigma-Aldrich, USA) for 15 min. Cells were washed with PBS and permeabilized with 0.2% Triton X-100/PBS. Nuclei and actin were stained with Hoechst 33342 and DY-488-Phalloidin (Dyomics GmbH, Germany) followed by confocal microscopy (Olympus FV-1000, Japan) at 200x. Initial image processing (background subtraction, contrast adjustment, maximum intensity projections) of the acquired z-stacks was performed with Fiji (NIH, USA).

Samples were briefly rinsed with Hank's buffered saline with 20 mM HEPES and fixed with 2.5% glutaraldehyde over night at 4°C. After fixation the samples were briefly rinsed with PBS before dehydration in an ascending ethanol series (70% - 80% - 96% - 100%) followed by a chemical drying procedure with hexamethyldisilazane. SEM was performed on a Quanta FEG 250 (FEI Company).

3 Results and discussion

3.1 Mechanical testing

The stress-strain curves obtained from uniaxial tension tests, illustrating the material behavior up to the ultimate tensile strength and point of failure, are presented in Figure 1. A summary of the mechanical parameters derived from these tests is provided in Table 1. For quantitative comparison, Young's modulus, ultimate tensile strength, and elongation at break were evaluated. Young's modulus was determined in the range 0% and 2%. Significant differences in tensile strength were observed among the cast films produced from various polymers.

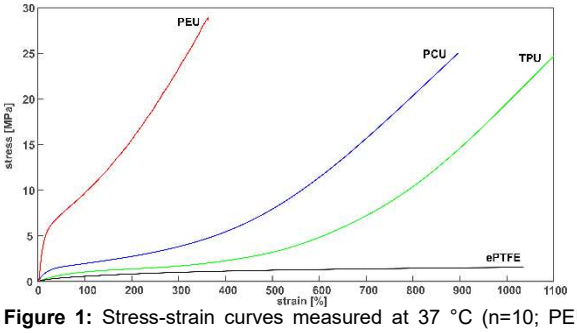

Figure 1: Stress-strain curves measured at 37 °C (n=10; PEU: polyether urethane, TPU: thermoplastic polyurethane, PCU: polycarbonate urethane)

ePTFE exhibited a low tensile strength of $\sigma = 1.12 \pm 0.05$ MPa and an elongation at break of $\varepsilon = 1035 \pm 17$ %. In contrast, the PCU and TPU samples demonstrated similar elongation before rupture but with marketly higher tensile strengths. While PEU displayed tensile strengths comparable to the other polyurethane samples, it had a notably lower elongation at break of approximately 325%.

Table 1: Mechanical parameters of polyurethane compared to ePTFE samples derived from uniaxial tensile tests. (PEU: polyether urethane, TPU: thermoplastic polyurethane, PCU: polycarbonate urethane)

	Young's modulus [MPa]	Tensile strength [MPa]	Elongation at break [%]
PEU	17.1 ± 1.5	28.9 ± 0.6	325 ± 16
PCU	3.7 ± 0.8	24.9 ± 2.1	927 ± 29
TPU	1.8 ± 0.9	25.1 ± 2.7	1103 ± 26
ePTFE	0.85 ± 0.13	1.12 ± 0.05	1035 ± 17

The differences in the mechanical behaviour of PU films can be attributed to the wide range of molecular structures and degrees of cross-linking. This results in material-specific fracture and elongation properties that require a targeted selection depending on the application. PU types with high strength and elongation properties could be particularly advantageous for dynamically stressed implants. At the same time, there are also differences in biological behaviour that preclude a general substitution of ePTFE. Further long-term studies on biostability and biological interaction are therefore essential.

3.2 Cytotoxicity testing

According to DIN EN ISO 10993 assessment of cytotoxicity of biomaterials is one of the first essential measurements with respect to biocompatibility of a biomaterial. Thus, all polymers were subjected to extraction in cell culture medium for subsequent treatment of cells according to ISO 10993-5. Cell viability measurement showed no difference in cell viability between the samples treated with pure medium (blind probe) and biomaterial extracts (Fig. 2). As for ePTFE (Zeus, USA) and the negative control (high density polyethylene, FDSC, Japan), all PU extracts exhibited cell viability values around 100% that is clearly above the cytotoxicity threshold of 70% (ISO 10993-5). Therefore, all polymer films were regarded as non-cytotoxic and subjected to microscopy analysis.

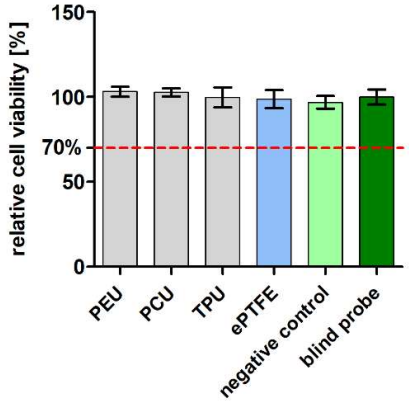

Figure 2: Cell viability of endothelial cells EA.hy926 24 h after treatment with biomaterial extracts. (MW ± SD, n=3; PEU: polyether urethane, TPU: thermoplastic polyurethane, PCU: polycarbonate urethane)

In order to evaluate the endothelialisation potential of the material, seeded polymer scaffolds were subjected to fluorescence microscopy and SEM. Cell morphology on the cover materials did not show any substantial differences among polyurethanes (Fig. 3). The cells mostly showed a morphology typical for endothelial cells and many contacts to neighbouring cells.

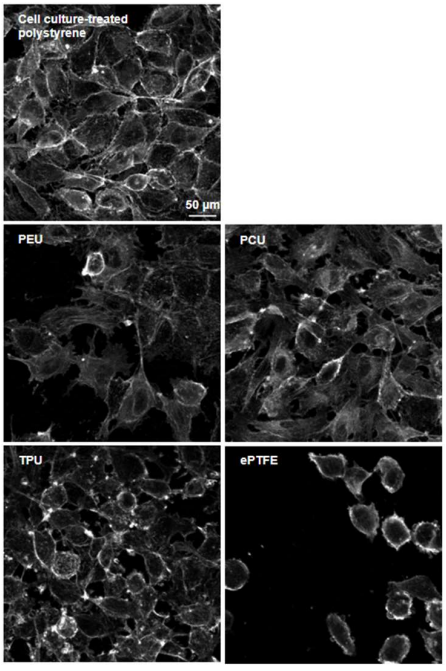

Figure 3: Fluorescent microscopy analysis of endothelial cell morphology (F-actin) when growing on PU-based biomaterials or ePTFE. (PEU: polyether urethane, TPU: thermoplastic polyurethane, PCU: polycarbonate urethane)

In contrast to cell culture polystyrene, however, gaps in the cell layer were observed in some areas of all polyurethanes examined, which are probably due to slower proliferation rate. Cell morphology on ePTFE was clearly different compared to the other materials. In contrast, there were fewer cells on ePTFE and their morphology was round, which is atypical for endothelial cells. Accordingly, the actin stress fibers were not formed. Therefore, ePTFE is not an optimal substrate for this cell type. This behaviour of cells on PTFE is known from in vitro studies and well documented in the literature [5].

Further insights into cell morphology on the materials were obtained via SEM analysis (Fig. 4). As shown in fig. 4 for polysterene, an optimized cell culture substrate, typical endothelial cell that form sufficient contacts to the substrate are flat and exhibit multiple contacts to neighbouring cells.

This corresponds to the typical morphology of endothelial cells in a physiological environment. Whereas a very similar picture could be observed for PEU and PCU, endothelial cells on TPU were in many cases relatively small. Many endothelial cells on TPU were round shaped and less flat, indicating distinct physicochemical properties of this PU substrate. For ePTFE, SEM analysis revealed a highly organized surface structure of ePTFE and only few round-shaped cells that were able to attach to its surface.

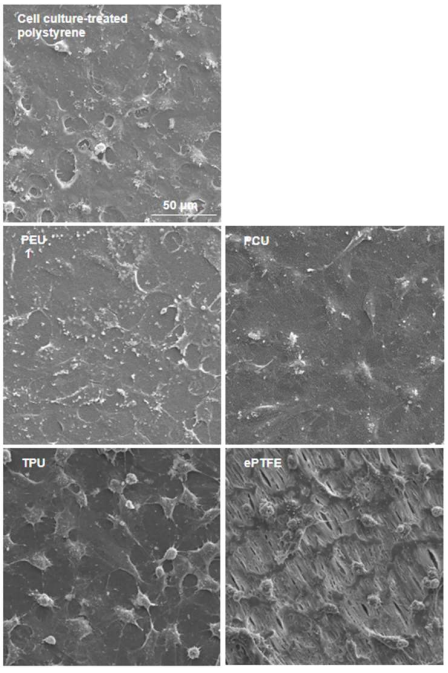

Figure 4: SEM analysis of endothelial cell morphology when growing on PU-based biomaterials or ePTFE. (PEU: polyether urethane, TPU: thermoplastic polyurethane, PCU: polycarbonate urethane)

4 Conclusion

The study demonstrates that polyurethanes present a promising alternative to expanded polytetrafluoroethylene. The tested PU films exhibited varying mechanical properties and distinct biological responses compared to ePTFE. These findings highlight the potential of different PU types while also indicating the need for further research, particularly regarding their long-term behaviour and biocompatibility. Furthermore, the variations among the different PU types underscore the importance of carefully selecting materials tailored for specific medical applications.

Author Statement

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