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RECYCLING OF LABORATORY PLASTIC WASTE – A FEASIBILITY STUDY ON CELL CULTURE FLASKS

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ABSTRACT: Laboratories contribute considerably to the global production of plastic waste. It was estimated that labs involved in biological, medical, and agricultural research produce around 5.5 million tonnes of plastic waste per year (Urbina et al. (2015) Nature 528, 479). Due to chemical and biological contamination, lab plastics pose a challenge for recycling and are most often used as single-use items. After use, these items are waste, and a recycling process or management system is missing. In cell culture, polystyrene (PS) vessels are predominantly used because of their favourable properties, such as transparency, stability, good cell-adhesive properties, and biocompatibility. This paper aims to present the recycling potential of the "laboratory plastic waste" using PS cell culture flasks, after decontamination by autoclaving, as an example. The different steps of the recycling process of the plastic material are presented as well as the biological evaluation of the recycled material. The mechanical and rheological characterization of the recycled PS parts shows a minimal reduction of material quality. The biological evaluation indicates that the recycled material does not meet the requirements of cell culture vessels. Further research is necessary to improve the transparency and surface-cell-adhesion properties of the parts made of the recycled material. Nevertheless, due to the good mechanical properties of the PS recycled material, it can be reused in other applications.

KEY WORDS: *Plastic waste; laboratory plastic waste; cell culture flasks; recycling.*

1. INTRODUCTION

Plastic articles are used throughout the world because of their beneficial features, such as durability, flexibility, and cost-effectiveness (Bennett and Alexandridis, 2021). This is why global plastic waste has increased tremendously within the last decades. Currently, 400 million tonnes of plastic waste are being produced every year (United Nations Environment Programme).

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Research laboratories make use of single-use plastics in many routine applications and thus strongly contribute to the growing amount of plastic waste. It is estimated that labs involved in biological, medical, and agricultural research produce around 5.5 million tonnes of plastic waste per year (Urbina et al., 2015). In recent years, individual scientific working groups have made attempts to reduce and recycle plastic waste (Alves et al., 2020; Howes, 2019; Reu et al., 2019). However, recycling contractors are often concerned about recycling of lab waste due to biological or chemical contamination (Howes, 2019).

In cell culture laboratories, particularly polystyrene (PS) cell culture vessels are used for cultivation of mammalian cells because of their favourable characteristics, such as transparency, stability, good cell-adhesive properties and biocompatibility. Moreover, manufacturing of PS vessels is relatively simple and cost-effective. To facilitate cell adhesion, PS surfaces can be easily modified by plasma treatment or coated with polymers or proteins (Lerman, et al., 2018). After usage, PS cell culture flasks are decontaminated by autoclaving and then disposed of as residual waste (Figure 1). In the past, cell cultivations were carried out in reusable glass bottles. However, due to the effort involved in cleaning and sterilizing glass labware, a return to this approach is not conceivable. Cell culture is a key technology in life sciences and indispensable in many areas of biotechnology and medicine, where it enables the production of biopharmaceuticals, tissue engineering or the cytotoxic assessment of chemicals without animal experiments. It is therefore assumed that PS plastic waste will even increase in the coming years.

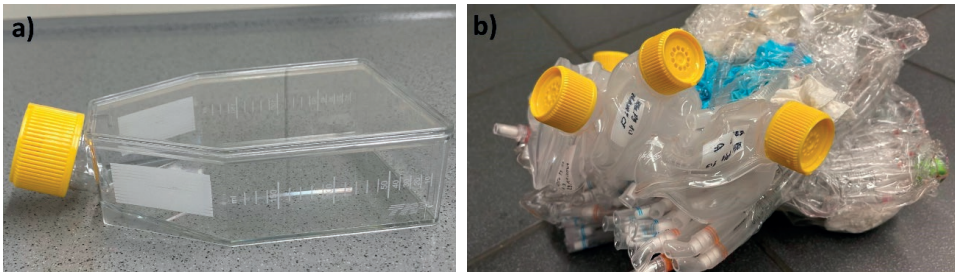

Figure 1. PS cell culture flask (a) and mixed, autoclaved lab waste with cell culture flasks (b).

The aim of this study was to set up a recycling process for autoclaved PS cell culture flasks in order to re-use the recycled material for the production of new cell culture vessels. Here, the different steps of the recycling process of the plastic material as well as results of material analysis are presented. To determine the characteristics of the recycled material for cell culture purposes, we performed cell culture experiments and subsequent microscopy with the standard cell lines CHO-K1 and 3T3-L1.

2. MATERIALS AND METHODS

Collection of cell culture flasks

For cell cultivation, T75 polystyrene cell culture flasks (TPP®) with a surface area of 75 cm^2 were used. After cultivation, empty flasks and caps were collected separately and autoclaved for 20 min at 121°C and 250 kPa (Varioklav 400 E, H+P Labortechnik).

Recycling process

Recycling was performed in three steps: separation (manual separation), shredding (moditec G1S ACP), injection moulding (Demag Ergotech 35-115).

Material analysis of PS

Polymer materials were identified by infrared spectroscopy (FTIR, Thermo Scientific Nicolet iS50). Samples of shredded PS for cell culture experiments and tensile testing specimens (1 BA ISO 527-2:2012) were prepared by injection moulding using a Demag Ergotech 35-115 with a clamping force of 35 tons. Characterization of mechanical behaviour of shredded PS was done using a tensile testing machine (Instron 4411 with 5 kN load cell) according to ISO 527:2012 with samples type 1 BA, where 5 samples were tested. Melt Flow Index (MFI) of PS samples was determined with Meltflixer ^{LT}. Microscopic evaluation was done with an inverted microscope (Avantor IT415PH). Images were taken using a camera (Bresser MikroCam PRO HDMI).

Cell culture

CHO-K1 cells (ATCC) were cultured in DMEM-F12 medium supplemented with 10 % (v/v) fetal bovine serum, 1 % (v/v) Penicillin/Streptomycin and 2 mM Ala-Gln. 3T3-L1 cells (ATCC) were cultured in DMEM (high glucose) supplemented with 10 % (v/v) neonatal calf serum, 1 % (v/v) Penicillin/Streptomycin and 20 mM Hepes. Cultivation was performed in a CO_2 incubator (Heracell 150, Thermo, 37 °C, 5 % CO_2). All components of the cell culture media were purchased from Sigma Aldrich.

Biological evaluation of PS materials

Plates (approximately 1 mm thick) of new and recycled PS were broken into smaller pieces of approximately 5 mm to 1 cm in diameter. For disinfection, the fragments were quickly rinsed in 70 % Ethanol and placed into Petri dishes in a laminar flow hood (Labgard ES Class II Biological Safety Cabinet). After ethanol was completely evaporated, PS pieces were transferred into 6-well plates (Greiner Bio-one). 4 ml of cells in culture medium ($5 \cdot 10^4$ cells/ml) were seeded on the different PS materials in 6-well plates and cultured for up to six days. For microscopic evaluation using an inverted microscope (Avantor IT415PH), samples were transferred into a new 6-well plate. Microscopy was performed at 100× or 200× magnification. Images were taken using a camera (Bresser MikroCam PRO HDMI).

3. RESULTS

Before setting up a recycling process, the plastic materials of unused T75 cell culture flasks (TPP®) were analysed visually (Figure 2) and by infrared spectroscopy (Table 1). The flasks consist of PS, whereas the yellow cap is made of high-density polyethylene (HD-PE). Moreover, the latter contains a polytetrafluoroethylene (PTFE) sealing/filter, a synthetic fluoropolymer that is used in numerous applications. To ensure proper recycling of the thermoplastic polymers, the separation of the different polymer fractions is necessary, as the combination of different types of plastics is a substantial obstacle to recycling (Sover et al., 2019, 2021 and 2022). Different companies work in this field of materials separation and provide good technical industrial solutions. In this study, the flasks and caps were separated manually to have a better understanding of the behaviour of each material after recycling and to avoid contamination with other materials. Here, separation was done for new cell culture flasks (Figure 2 a) and flasks collected after having been used with cultured cells and autoclaved (Figure 1 b).

Table 1. FTIR identification of the materials from PS cell culture flasks.

component of cell culture flask	FTIR analysis		
	material	probability [%]	weight [g]
flask	PS 454 H	98.57	43.49
cap	HD-PE BorPur MB 7541	92.17	3.98
filter for ventilation	PTFE	71.81	
printing	Butyl benzyl phtalate	70.13	

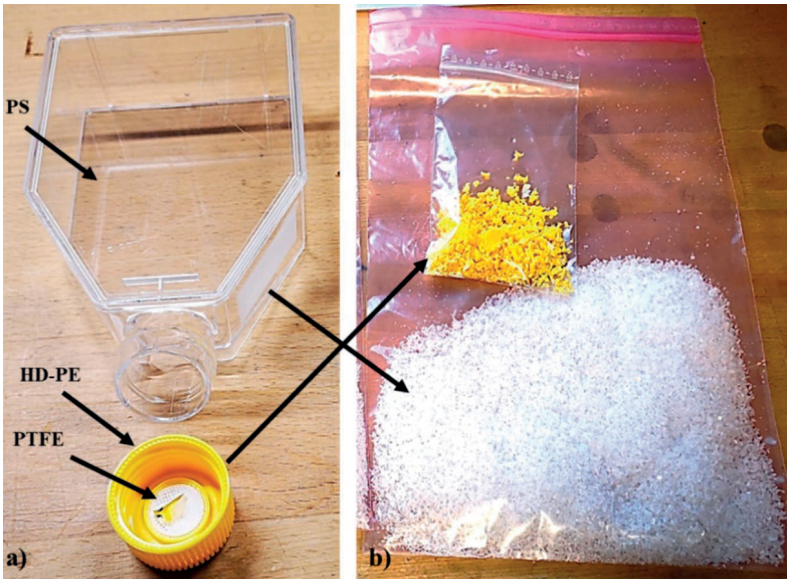

Figure 2. Cell culture flask, components and materials (a) and granulated components (b).

Recycling was performed in three steps: separation, shredding and injection moulding. As cell culture materials are usually autoclaved at high temperatures (121 °C) and high pressure (approximately 250 kPa) for biological decontamination, we aimed to find out about the impact of this harsh treatment on the plastic materials. For this purpose, the recycling process was compared for new (unused) and used (autoclaved) cell culture flasks. After shredding, samples were prepared by injection moulding in order to assess polymer quality regarding degradation. This evaluation was carried out after the mentioned processing steps which are state of the art in different recycling companies.

The processing of the PS flask did not cause any problems following the recommendation of parameters for PS polymer. 60 mm x 60 mm plaque samples were produced to inspect the transparency properties of the polymer material after the recycling process and for later biological evaluation. Moreover, tensile testing specimens (1 BA ISO 527-2:2012) were prepared by injection moulding (Figure 3).

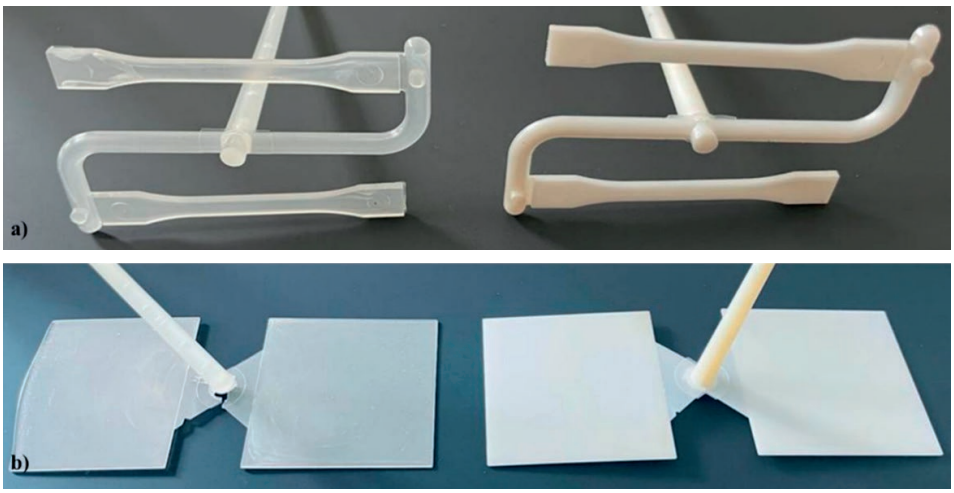

Figure 3. Tensile testing samples (a) and 60 × 60 mm plaques (b) of PS materials from the new (left) and used (right) flask prepared by injection moulding.

After processing, the properties of recycled material were analysed and compared to new material. Validation of Melt Flow Index (MFI) indicated no major differences between the two types of materials as MFI of the polymer from the used and shredded flasks was 2.9 g/10 min (ISO 1133, 2.3 kg, 190°C, measured with Meltflixer ^{LT}) while the MFI of the original flasks was 3 g/10 min.

Characterization of the mechanical behaviour of the shredded new flasks and used flasks was performed using a tensile testing machine according to ISO 527:2012 with samples type 1 BA, where 5 samples were tested. The results are presented in Table 2.

Table 2. Results of tensile testing of shredded PS polymer.

parameters	tensile testing results (ISO 527)	
	new flasks	used flasks
max. Force (Fmax) [N]	560	530
E-Modulus [MPa]	1337	1352
tensile strength (Rm) [MPa]	48.29	46.63

Also here, observed differences in the mechanical properties of the shredded PS polymer for new flasks and used flasks were minimal (under 5 %), which implies that the polymer material does not lose its mechanical characteristics after autoclaving.

Visual comparison of recycled PS material with new, not recycled material revealed that transparency is evidently lower after recycling (Figure 4). This obvious effect can be a problem for re-utilisation of the PS recycled polymer in the production of cell culture flasks, considering that, in cell culture, cell morphology and proliferation is usually inspected by transmitted light microscopy. Moreover, during microscopic evaluation of the recycled material, it was not possible to adjust the sharpness on the microscope, probably due to surface characteristics (Figure 4 d).

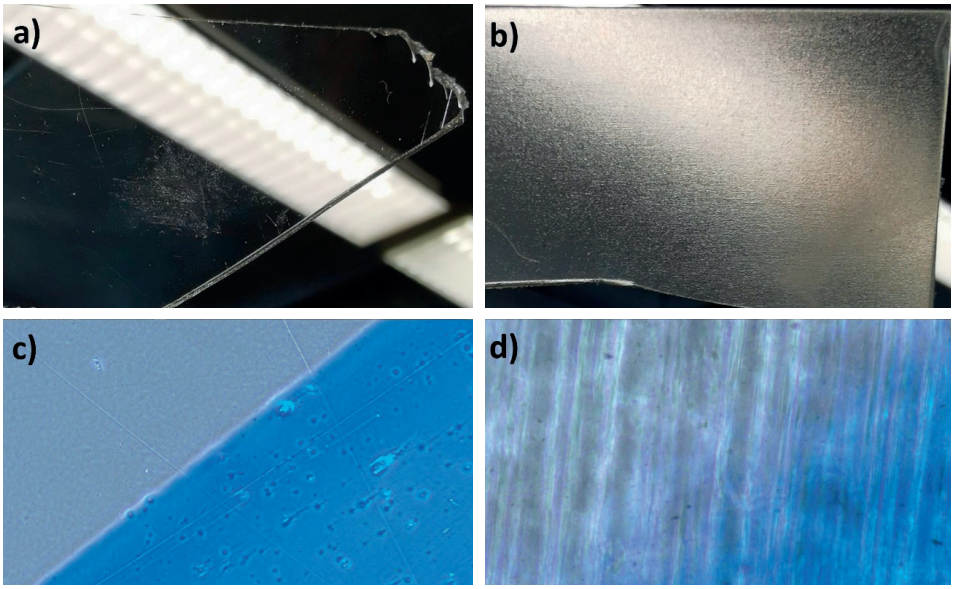

Figure 4. Visual comparison of transparency of new cell culture flask (a) and recycled material (b) and microscopic inspection of the surfaces (c: new PS; d: recycled PS). To adjust sharpness on the microscope, a blue line was drawn on the materials.

In their natural environment, the majority of mammalian cells attach to an extracellular matrix. In the artificial setting of cell culture, they adhere to the surface of a cell culture vessel instead. In order to determine the biocompatibility of the recycled PS, two different cell lines were seeded on small plates of new and recycled PS and microscoped after six days of culture (Figure 5). On the original PS material, CHO-K1 cells adhered and grew to confluence, showing an epithelial-like morphology. Adherence was also observed for 3T3-L1 cells exhibiting typical spindle-shape morphology. In contrast, on recycled PS, microscopy was complicated because of the cloudy material surface. However, discrete shapes of cells were detectable, although at decreased number. It is therefore assumed that cell-adhesive properties of recycled PS are reduced.

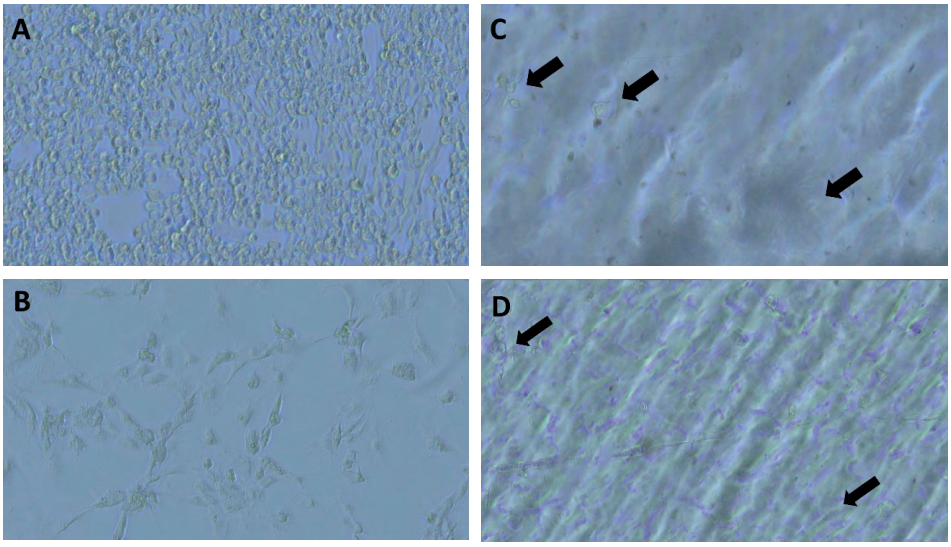

Figure 5. Biological evaluation of new cell culture flask and recycled material. CHO-K1 (a, c) and 3T3-L1 (b, d) cells were grown for six days on different materials in 6-well plates. On new cell culture flasks (a, b), cells adhered and showed typical shape. On recycled material (c, d), cells are not clearly visible. CHO-K1: 200× magnification; 3T3-L1: 100× magnification. Discrete shapes of cells are exemplarily indicated by arrows.

4. CONCLUSION

Our investigation shows that the recycling of cell culture flasks is possible with a minimal decline of mechanical and rheological properties of the recycled PS. The decrease in transparency is substantial and affects the potential re-use of this material for cell culture purposes. However, with the aid of additives and changes of process conditions, transparency could be enhanced (Guzman-Puyol, et all., 2022). So far, the important feature of good cell adhesion was not fully achieved by the recycled polymer. This property could be improved by surface modifications, e.g., plasma treatment (Lerman, et al., 2018).

However, the recycled PS polymer promises to be readily usable for other (non-cell culture) applications, e.g. white goods and automotive, where the mechanical properties are more important than biocompatibility and microscopic properties of the material. For those non-cell culture applications, it is essential to ensure the safety of the PS polymer. This is even more important as some cell culture labs work with hazardous biological organisms or genetically modified cell lines. During autoclaving, those organisms are killed. However, it was shown that DNA can resist denaturation at high temperatures (Wang et al, 2014). Thus, our future efforts aim to confirm that the recycled material is free of DNA from previously cultured cells to make it usable for non-cell culture purposes.

Overall, the price-benefit of the recycled PS polymer can be interesting for recycling companies. This might represent a good step towards a cycle economy helping to re-use plastic material and act environmentally responsible.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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