Research Article

3D-printed individual labware in biosciences by rapid prototyping: *In vitro* biocompatibility and applications for eukaryotic cell cultures

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Abbreviations:

ABS	acrylonitrile butadiene styrene
CAD	computer-aided design
CTB	CellTiter-Blue®
FDM	fused layer depositioning
LDH	lactate dehydrogenase
PA	polyamide
PLA	polylactic acid
ROS	radical oxygen species
RP	rapid prototyping
SLS	selective laser sintering
SU	single-use
USP	U.S. Pharmacopeia

Practical application

Individual labware created with 3D-printers directly in a laboratory will advance science and research in the near future. As an example a specifically designed well plate is used to illustrate this approach. Using 3D-printing technologies it will be possible to adapt the labware design to fulfil specific requirements of an experiment or an organism that is being cultured, instead of fitting a broad range of needs to standard labware design. Furthermore the 3D-printing material was examined for their applicability within a biotechnological laboratory. The expiration of patents protecting specific 3D-printing technologies and the fast paced advancements will make this technology available for many laboratories around the world within a couple of years.

Abstract

3D-printing techniques are continuously evolving, thus their application fields are likewise growing very fast. The applications shown here highlight the use of rapid prototyping (RP) in a dedicated biotechnology laboratory environment. The combination of improving prototypes using fused deposition modeling (FDM) printers and producing useable parts with selective laser sintering (SLS) printers enable a cost- and time-efficient use of these techniques. Biocompatible materials for 3D-printing are already available and the printed parts can directly be used in the laboratory. To demonstrate this, we tested 3D-printing materials for their *in vitro* biocompatibility. To exemplify the versatility of the 3D-printing process applied to a biotechnology laboratory, a normal well plate design was modified *in silico* to include different baffle geometries. This plate was subsequently 3D-printed and used for cultivation. In the near future this design and print possibility will revolutionize the industry. Advanced printers will become available for laboratories and could be used for creating individual labware or standard disposables on demand. These applications have the potential to change the way research is done and even how stock-keeping management is today, leading to more flexibility and promoting creativity of the scientists.

1. Introduction

Nowadays the development of new components for the use in biotechnology laboratories, like simple disposable labware, is very time consuming and adequate manufacturing installations and methods are needed. The design and manufacturing of new labware and new applications of these is relegated basically to the big companies. One recent example is the appearance of a 5 mL Eppendorf tube. Development and manufacturing of prototypes are complex processes comprising multiple steps to obtain a fully functional end product. The fabrication difficulties are explained using the design and construction of a modified baffled well plate for increasing the oxygen intake over the normal designs. For the high quality fabrication of well plates an appropriate casting mold has to be created and used as a tool for a further deep-drawing process. FUNKE ET AL. used laser cutting to cut the well geometries out of acrylic glass plates [1]. This step already presents multiple drawbacks when it comes to usability of the prototypes. For instance it is only possible to process certain material thicknesses with laser cutting machines and furthermore just few materials allow a focusing of the laser beam [2]. After this step a bottom-plate had to be glued under this geometry-plate to seal it completely. In this example it was done via gluing, which itself leads to multiple problems. The glue needs to completely seal the geometries without creating trapped areas or hard to clean gaps.

The glue used must withstand sterilization, which can include irradiation or steam-sterilization and needs to be biocompatible.

All these problems and the multi-step building-process can be simplified and accelerated with the use of 3D-printing. The same scenario of the well plate construction is not only simplified, but design possibilities are extended. Modifying the wells individually, e.g. define rounded corners on the bottom or even geometry differences within the height of a single well. The plate is still created in one solid piece. This would not be possible using 2D-laser cutting techniques. To create this 3D-printed well plate, first it needs to be digitalized into a 3D-model using CAD-software. The obtained 3D-model must be prepared to be printed, which is done by digitally slicing the model into the different 2D-layers. This is done via specific software, usually proprietary, depending on the printer to be used. The printer used for printing the laboratory labware is based on the SLS-method, in which a polymer-powder is spread across the printer's building platform forming a flat surface, where one slice of the model will be printed by melting (sintering) the polymer with a focused LASER. After the slice is printed the process is repeated adding layer over layer until the complete model is finished. To be able to benefit from 3D-printing in the area of biotechnology it is fundamental that the used material meets several requirements. These include chemical stability and biocompatibility, as well as resistance to temperature and radiation for sterilization purposes. In this article we focus on biocompatibility and sterilization resistance, as compliance with these two will yield a usable device for the general biotechnological use.

To assess the biocompatibility of our 3D-printed prototypes several tests were conducted. The first experiments were to test the raw material itself, the norm ISO 10993-12:2012 was followed to obtain an extraction media which was tested on several adherent and suspension cell lines [3, 4]. On another experiment adherent cell lines were directly cultivated in the printed well plates.

Having shown general biocompatibility we proceeded to demonstrate the potential of 3D-printed individual labware with the example of a completely printed baffled well plate. This well plate contains wells of different geometries and design. These modifications are meant to modify and increase the oxygen intake in the culture media over a normal round-shaped well. Such a well plate could be used to study an organisms behavior in different oxygen concentration conditions in parallel due to the individual differences in k_La obtained by the baffle design. Another use could be the evaluation of mixing performance due to the modification of the wells. To demonstrate the usability of this well plate and the effect of these geometries we cultivated *S. cerevisiae* as a eukaryotic model organism. It was selected due to its higher oxygen consumption and faster growth in comparision to mammalian cells. By using a high oxygen demanding microorganism, growth rate

can be used as an indication of oxygen limitation and correlated to the oxygen transfer created by the different well geometries.

This well plate is just one example used to highlight the possibilities of 3D-printing in a biotechnological laboratory. Scientists face only their imagination as a limit and can now create new labware designed especially for their needs. A proof of principle with different practical examples is also available [5].

2. Materials and methods

Modelling and printer technology

All models for 3D-printing were created with the computer aided design (CAD) software Autodesk Inventor Professional 2014 (Autodesk, Inc., San Rafael, California, USA).

For the construction of the new prototypes two different 3D-printing techniques were used, FDM (fused deposition modelling - the term FDM is trademarked by Stratasys Inc.) and SLS (selective laser sintering).

Printing with the FDM-technique was done with a desktop oriented, low-priced CubeX Trio printer (3D Systems, Rock Hill, South Carolina, USA) [6]. This printer enabled us to get the first physical models and optimize geometries which suited our needs. The materials available for this printer are polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS). Unfortunately these materials are not suitable for laboratory use.

Printing with the SLS-technique was done by Blue Production (Paderborn, Germany). With this printer-system the material used was polyamide (PA) 12 powder [6]. Models created with this technique are potentially suitable to use in the laboratory environment when it comes to chemical stability, biocompatibility and withstanding sterilization conditions.

3D-printing raw materials and preparation of extraction media

The SLS 3D-printed well plates and the unprocessed polyamide powder were obtained from Blue Production (Paderborn, Germany). Two different types of polyamide were used, polyamide 12 (in the following referred to as PA12) and the polyamide 12 combined with an undisclosed UV-stabilizer (in the following referred to as PA12 UV). For the biocompatibility experiments an extraction medium was created according to ISO 10993-12:2012 (Biological evaluation of medical devices – Part 12: Sample preparation and reference materials) [4]. Therefore the powder was sterilized by autoclaving (121 °C, 2 bar, 30 min).

The culture medium appropriate for each cell line (see below) was mixed with the polyamide powder (0.2 g/mL). These media-mixtures were placed in an incubator at 37 °C for 72 h at a shaking speed of 160 rpm. To remove the powder the media was centrifuged at 3,000 x *g* for 1 h (Centrifuge 5702, Eppendorf AG, Hamburg, Germany). The supernatants were filtered using a 0.22 µm sterile filter (Filtropur S, Sarstedt, Nuembrecht, Germany). The obtained extraction medium was used for the cultivation experiments.

3D printed well plates

For the direct cultivation experiments two different designs of well plates were modelled, type A and B, and 3D printed with a selective laser sintering (SLS) machine out of the PA12- and PA12 UV-material. Type A is an exact replica of a commercially available 24-well plate (Culture Plate Flat Bottom, Sarstedt, Newton, NC, USA), it was used for the biocompatibility experiments using adherent mammalian cells.

Type B is a custom-designed well plate with 23 different well-geometries. This type B design was used for the culture of yeast cells. [1] A rendered CAD-image (A) and the printed model (B) of the well plate type “B” are shown in Figure 1.

Cell culture and cell treatment – Adherent cells

The raw material biocompatibility tests were carried out in standard 24-well plates, 1.82 cm² growth area, approximately 1 mL maximum working volume (Culture Plate Flat Bottom, Sarstedt, Newton, NC, USA). Two different adherent cell lines were used, a human epithelial lung cell line A549 (DSMZ no: ACC 107), and mouse fibroblasts NIH-3T3 (DSMZ no: ACC 59). Both were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma Aldrich, St. Louis, MO, USA) supplemented with 10 % fetal calf serum (Biochrom GmbH, Berlin, Germany) and 1 % (v/v) Penicilin/Streptomycin (PAA Laboratories GmbH, Pasching, Austria). A549 and NIH-3T3 cells were seeded at a density of $1.5 \cdot 10^4$ cells/cm² and a final volume of 300 µL and maintained at 37 °C with 5 % (v/v) CO_2.

The normal culture media was removed from the 24-well plate after 24 h and extraction media was added for another cultivation period of 24 h.

To test the actual 3D-printed well plates, cells were cultured in the printed well plate “type A” for 48 h. A standard 24-well plate cultivation was run in parallel for reference.

In both cases after the cultivation period was over, the cell count was measured and the CellTiter-Blue® (CTB) cell viability Assay and the CytoTox-ONE™ Homogeneous Membrane Integrity Assay (LDH) were performed.

Cell culture and cell treatment – Suspension cells

Suspension adapted Chinese hamster ovary cells CHO-K1-HP (kindly provided by AG Cell Culture Technology, University of Bielefeld, Germany) were maintained in shaking flasks using CHOMACS CD media (Miltenyi Biotec, Bergisch Gladbach, Germany) supplemented with L-glutamine (8 mM, Biochrom, Berlin, Germany).

Raw material biocompatibility test was done using CHO-K1-HP cells cultured in extraction media and untreated media (as reference) in 250 mL shaking flasks (Corning, Tewksbury, MA, USA) maintained at 37 °C, 5 % CO_2 (v/v) and 160 rpm for 5 days starting with a cell density of $4.5 \cdot 10^5$ cells/mL.

CellTiter-Blue® (CTB) cell viability assay

To determine the cell viability of A549 and NIH-3T3 cells the CellTiter-Blue® (CTB) cell viability assay (Promega, Mannheim, Germany) was used. Living cells are able to convert resazurin into resorufin, which fluoresces. Necrotic cells however are not able to reduce resazurin and therefor are not generating the fluorescent product.

After the incubation period the CTB-assay was performed as instructed by the manufacturer, including background and control groups. At first the medium was removed from each well and 250 µL of fresh medium containing 10 % (v/v) of the CTB stock solution were added to each well and incubated for 2h. The fluorescence of resorufin reduced by viable cells is measured at 544/590 nm with a fluorescence plate reader (Fluoroskan Acent, Thermo Fisher Scientific Inc., Waltham, USA).

CytoTox-ONE™ Homogeneous Membrane Integrity Assay (LDH)

The CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega, Mannheim, Germany) was used to determine the membrane integrity of cultured cells. Through the loss of membrane integrity the enzyme lactate dehydrogenase (LDH) is released from the cells into the surrounding culture medium. The amount of leaked LDH is measured by the conversion of added resazurin into resorufin using fluorescence measurement at 544/590 nm. The amount of leaked LDH is proportional to the number of non-viable cells and can be used to calculate the percentage of a cytotoxic effect. The assay can also be used in combination with a lysis solution, to get an estimation about the total cell count. The LDH-assay was performed with A549 and NIH-3T3 cells. Cells were seeded as described above. For the determination of cell death 100 µL medium from each well were transferred into a new 96-well plate. For calculation of cell count after exposure cell lysis was induced before collecting the culture media. Both variants of the protocol, with and without cell-lysis, were used for the experiments. Afterwards the LDH-assay was used as instructed by the manufacturer.

Measuring cell proliferation (cell counting)

For the adherent cells the cell proliferation was measured by determination of the cell count using an improved Neubauer chamber. Cells were cultured as described before. After the cultivation media was removed, cells were detached from the well plates using 0.2 % Trypsin/0.02 % EDTA solution (approx. 5 min, 37 °C, 5% (v/v) CO_2).

For the suspension cells CHO-K1-HP proliferation and viability was measured using the cell counter Cedex (Roche Innovatis AG, Penzberg, Germany). During the 5 day incubation period (see above) the measurement was performed twice a day.

Cultivation of Saccharomyces cerevisiae

Yeast media (YM) containing 3 g/L yeast extract, 3 g/L maltose and 5 g/L soy broth dissolved in deionized water was used. The pH value of the medium was adjusted to approximately pH 5.5. After autoclaving, 10 g/L glucose (separately sterilized) was added. For the *Saccharomyces cerevisiae* pre-culture a colony was taken from an agar plate and suspended in a 250 mL shaking flask containing 100 mL yeast medium. The culture was incubated at 30 °C on a shaker at 220 rpm for 24 h. For the experiment the optical density (OD) of the culture was measured at 600 nm. The OD was adjusted to $OD_{600\ nm}$ = 0.5 by mixing with fresh culture medium in a new flask. For the cultivation in the 3D-printed well plates “type B” (PA12 and PA12 UV) 1,000 µL of prepared yeast culture was seeded into each individual well. The well plates were incubated for 24 h at 30 °C and shaken at 175 rpm. After the incubation time the optical density of each well was measured at 600 nm using a spectrometer (Multiskan™ GO, Thermo Scientific, Waltham, MA, USA).

3. Results and discussion

Every rapid prototyping technology has its own advantages and disadvantages. Especially the demanding requirements for the use with mammalian cell culture narrow the choice of the possible printing methods. The most promising method is SLS-printing, because it has the broadest range of usable materials. However, one of the main issues with the SLS-printing technology is that sintered materials tend to have a rather porous structure [7]. The chosen manufacturer optimized the sintering method together with the material composition to achieve minimal porosity. During experiments we observed that a complete leakproofness depends on the sterilization method. Although the material is able to withstand standard steam sterilization methods (121 °C, 2 bar, 30 min) without observable changes, the 3D-printed parts becomes more porous. Furthermore, if the

material gets in contact with alcohol, e.g. 70 % isopropanol or ethanol for cleaning purposes, the volatile solutions swell the structure, leading to leaking of the liquids. When it comes to the use of RP for generation of scaffolds for tissue engineering this porosity can be a desired trait, but for creating culture vessels this is a drawback. Different approaches to sterilize the well plates were used. For the experiments with yeast it was sufficient to irradiate the plates with UV-light (HERAsafe KS, Thermo Electron, Langenselbold, Germany, 10 cm distance, 60 minutes) and achieve thereby a germ-poor environment. For the use with mammalian cells, sterilization with gamma-irradiation was chosen as the standard technique that is already widely used for labware and especially single-use equipment and bioprocess systems [8, 9]. With the use of irradiation-sterilization the created parts are not physically altered and can be used directly for cultivation purposes.

The leak-tightness is still a problem that has to be faced in further optimization steps. This can be done by improving fabrication parameters, e.g. the sintering-time by the LASER-beam or by adding a post-processing of the printed parts. This could be an infiltration with a suitable and also biocompatible material, e.g. a resin that would be vacuum-impregnated into the part using a desiccator and subsequently completed in a curing-oven.

Adherent cell cultivation in extraction media

As model organisms for the biocompatibility testing, epithelial cells (A549) and fibroblasts (NIH-3T3) were cultivated. To detect the possible toxic effects the cells and the supernatant were tested with different assays for adherent cell cultivations. The used methods were a CTB-assay and LDH-assay. The first one gives evidence about the vitality measured by the metabolic activity of the cells and the second one gives a statement about the amount of necrotic cells. An additional manual cell counting was carried out.

Results from the CTB-assay are summarized in

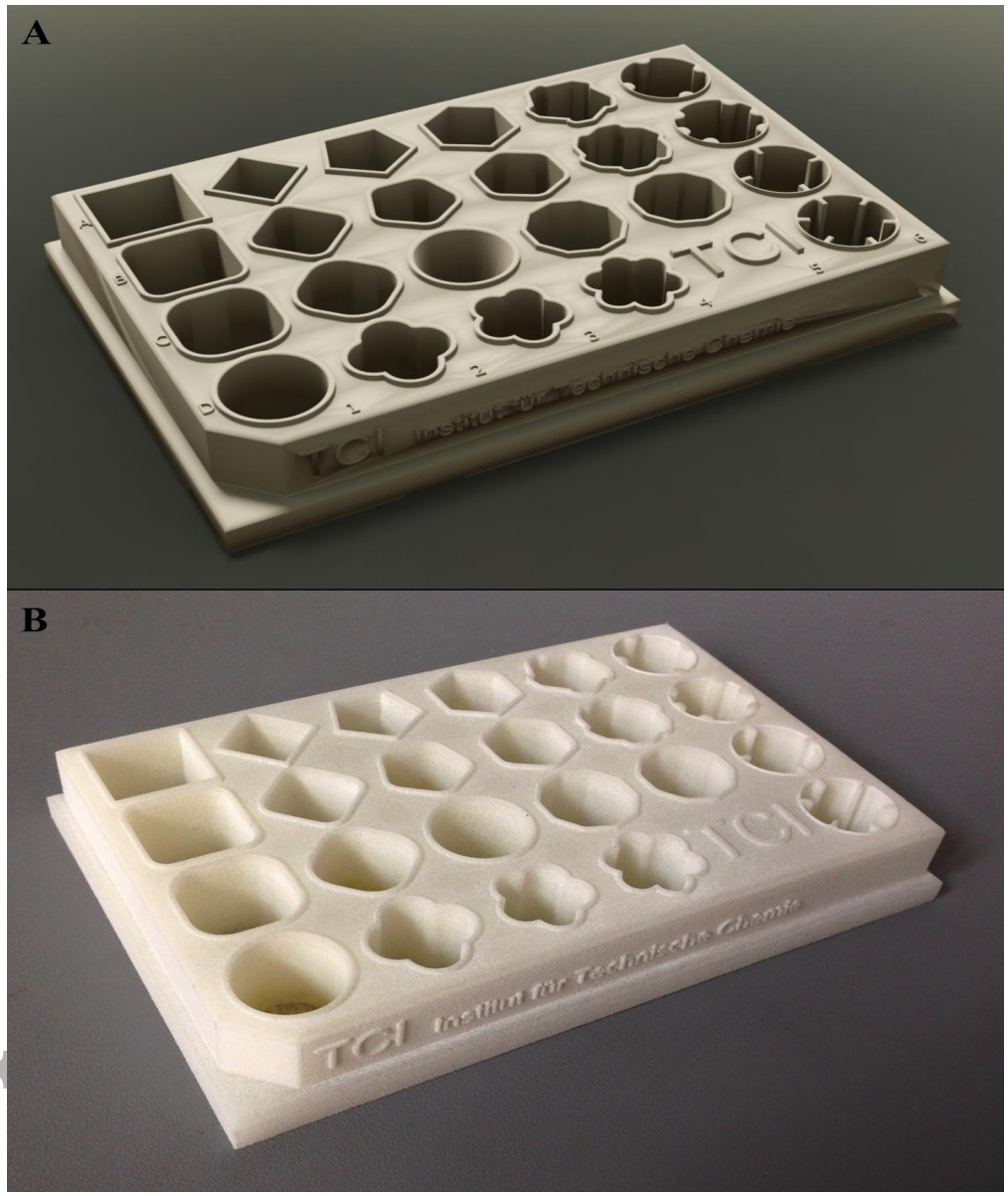

Figure 2.

The CTB test indirectly measures the metabolic activity of the cells and thus gives an indication of the cell vitality. Both cell lines show the same behavior after being in contact with the extraction media for one day. Compared to the reference cultures both experiments with extraction media show a slightly improved metabolic activity. The comparison of the PA12- and PA12 UV-extraction media cultivations shows no large variations among them.

Cell count of the same experiment is shown in

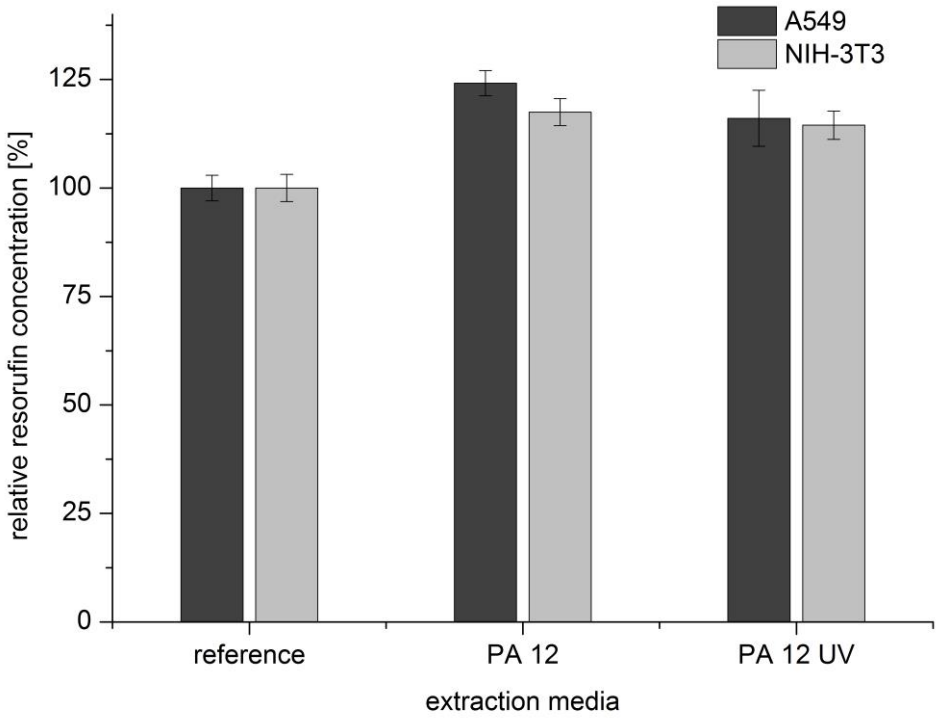

Figure 3. All cultures reach a relative similar concentration. Both cultivations with extraction media present the same behavior. They show a small deviation in cell count.

The LDH-concentration set free by cell lysis is shown in

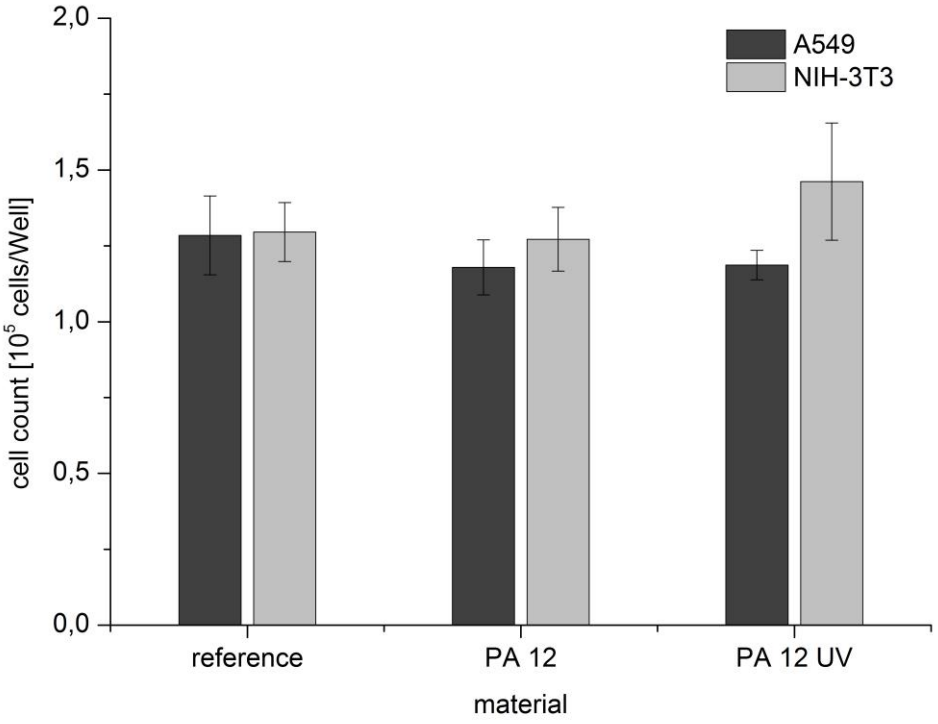

Figure 4. The results show a slightly lower LDH-release and thus cell count with the PA 12-extraction media and a slightly higher cell count with the PA 12 UV-extraction media. This effect is more visible in the experiments with NIH-3T3 cells. In our experiences NIH-3T3 cells are always more sensitive to toxic effects compared to A549 cells [10].

Adherent cultivation in printed 24-well plates

In addition to the extraction media experiments, a cultivation directly in a printed 24-well plate (well plate "type A") was carried out. These experiments will test a more prolonged and intense contact of the cells and the material. It will also reflect any discrepancy between raw material and processed 3D-printed material properties regarding biocompatibility.

This well plate was modelled as an exact replica of the used standard well plates, which at the same time was used as a reference. Because of the initial results and the already known biocompatibility of the PA 12-material, this experiment was carried out only with the well plate made of PA 12 UV-material.

The surface of the printed well plate is rougher in comparison to a commercial well plate, this made detaching the cells from the surface very difficult. For that reason only the LDH assay for determination of cell death, without cell-lysis, was performed. The results for the LDH-release are shown in

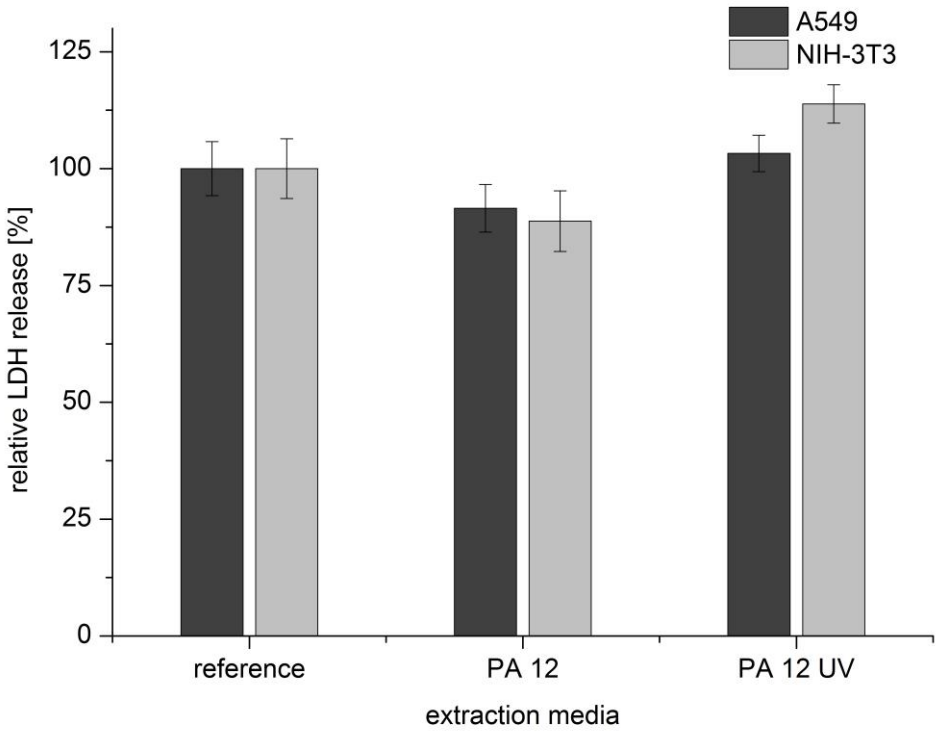

Figure 5. It shows that the reference and the well plate “type A” made of PA12 UV have the same amount of active cells after the cultivation period. This is the case for the A549 cells, as well as for the NIH-3T3 cells.

CHO-K1-HP suspension cultivation

For the biocompatibility testing with suspension cells, CHO-K1-HP were cultured in shaking flasks with the prepared PA12- and PA12 UV-extraction media. The growth curves were compared to cells cultivated in untreated media to see the effect of possible extractables or leachables out of the material. Since the cells grew in suspension and a higher volume of cell suspension was available, the culture condition could be measured using an automated cell counter. Therefore it was not necessary to use the above mentioned assays. The results of the cultivations are shown in

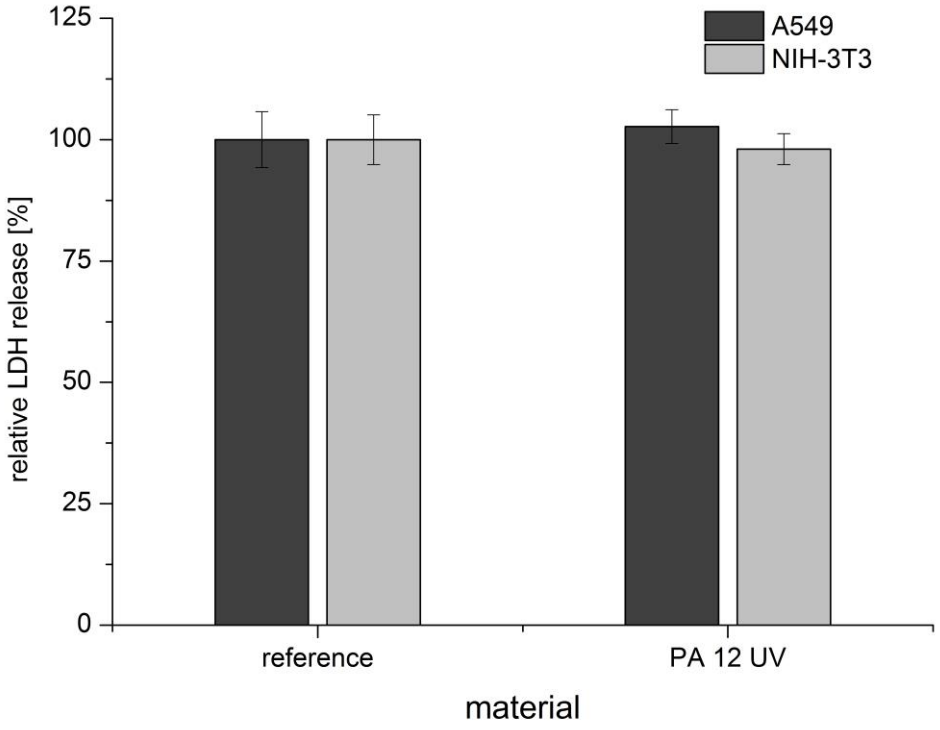

Figure 6.

The results show two different growth behaviors. All flasks started with cell concentration of $4.5 \cdot 10^5$ cells/mL. The extraction medium, which was created with the PA12 UV-powder, resulted in the best growth behavior. Compared to the cultivation with PA12-extraction medium and the reference, the cell concentration after 92 h was almost two times higher (1.96 times higher). The reference cultivation started with a slightly lower cell growth, but caught up after three days. Taking this into account the cultures with reference media and the PA12 extracted media have similar growth behavior and thus endorse the biocompatibility of this material. Therefore these results confirm the U.S. Pharmacopeia (USP) biocompatibility certification of the PA12-material. The experiments with the extracted PA12 UV-media also support the biocompatibility of the material. Furthermore it shows unexpected promises in other aspects, like the observed increasing cell number.

To achieve the UV-stability of the improved PA12-material an undisclosed amount of an antioxidant was added to the powder. Antioxidants have in general a stabilizing effect on cells. This supports the assumption that oxidative stress is a major problem in cell cultures and that medium is often not sufficiently supplemented with antioxidants [11–13]. This could explain why the cultures treated with this antioxidant enriched medium have a higher proliferation than the others. The proprietary composition of the used media, CHOMACS CD, seems to be insufficiently provided with proper antioxidants.

The comparison between the adherent cells cultivated in well plates and the suspension cells cultivated in shaking flasks

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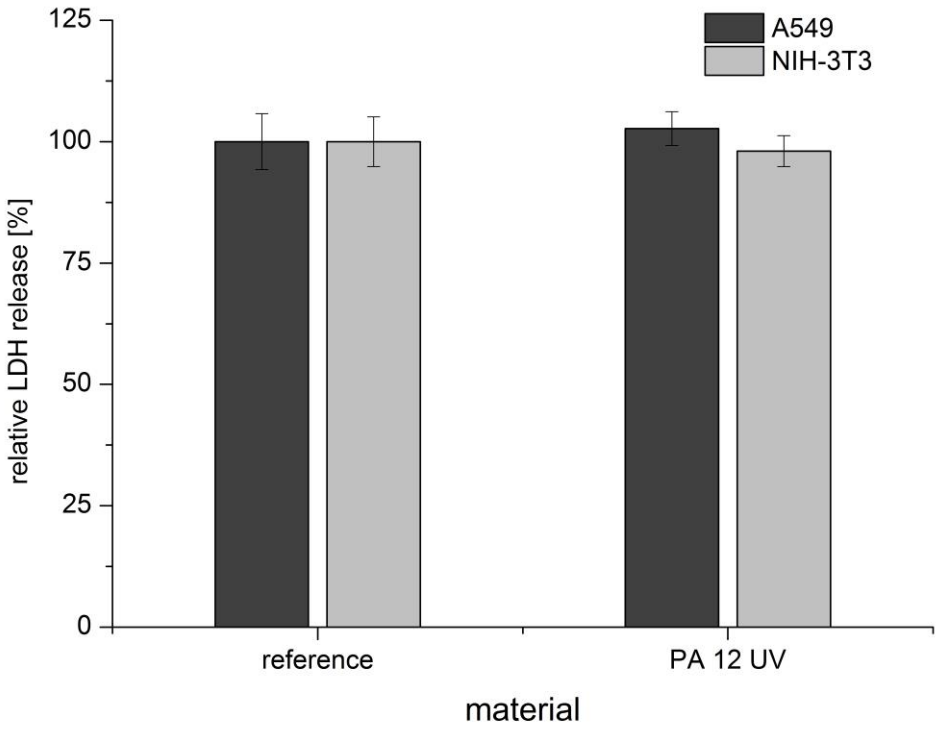

Figure 6) show that there was no strong positive or negative effect within the adherent cell experiments. We assume that the reason is the reduced oxygen diffusion in the stationary well plates compared to the orbital shaken flasks. The bigger oxygen transfer in the flasks could have resulted in larger amounts of radical oxygen species (ROS) being formed in the culture media. Therefore the positive effect of the added antioxidant could be more easily observed. The absence of ROS in the stationary well plates could explain why those results show no great difference between the PA 12- and PA 12 UV-material.

Nevertheless the PA 12 UV-material shows equal and in some cases even better results, than the already USP-certified PA 12-material. This backs up the use of the novel UV-stable material for certain applications, and supports the biocompatibility of the standard material.

Yeast cultivation in baffled well plates

After showing that the material is biocompatible and suitable for cultivation purposes, an application example was tested.

A baffled well plate with 23 different well-geometries within one well plate was designed and 3D-printed (well plate type “B”). In contrast to previous publications of other work groups we simplified the fabrication drastically and created the well plate as one solid piece all in one step [1].

For this experiment we chose *S. cerevisiae* as a eukaryotic model organism with quick growth and a higher oxygen consumption.

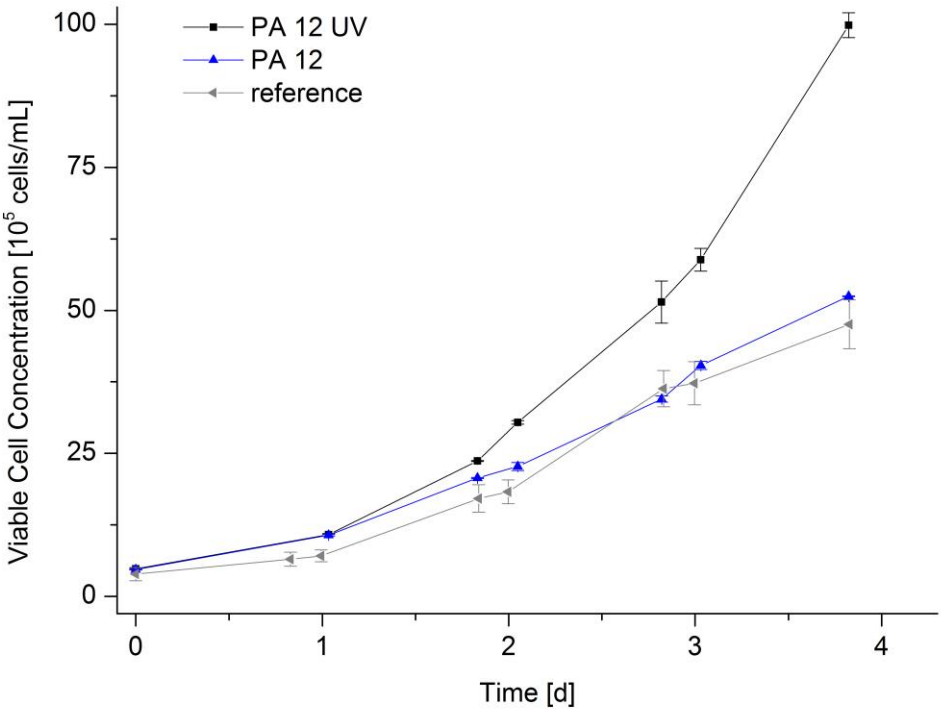

Figure 7 shows the results of four different well geometries, starting from the square-shaped well and increasing the roundness of the corners until a completely round well is achieved.

We observed the highest growth after 24 h in the square-shaped well. Optical densities decreased with increasing corner radius of the well-geometry (

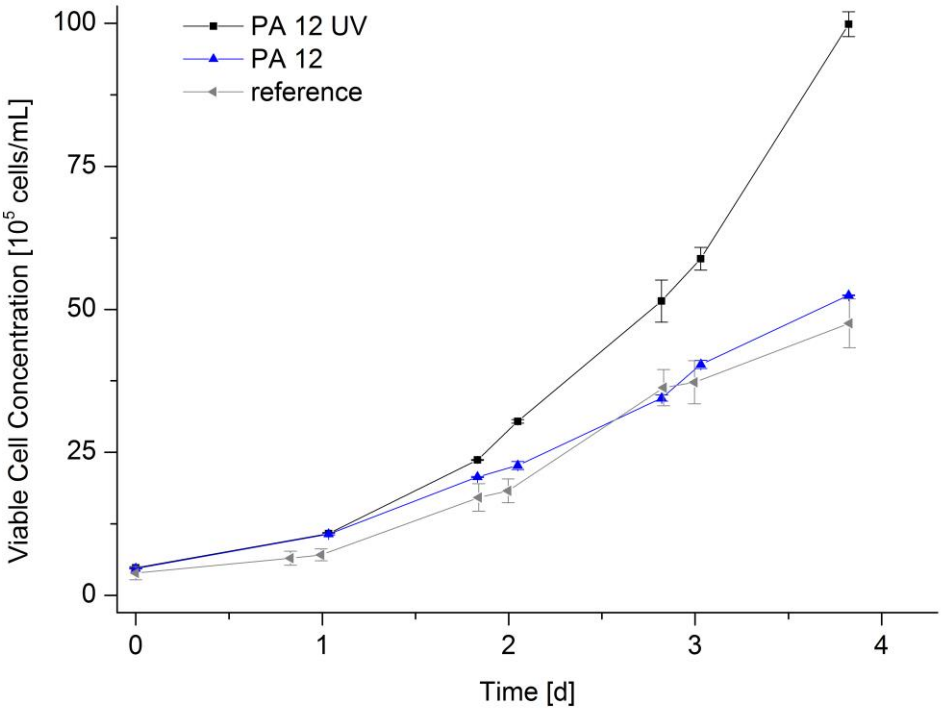

Figure 7).

The results illustrate the effect of including edges in the well geometry. Their inclusion increases the turbulent mixing and thus lead to a higher oxygen intake. This corresponds with the observations of FUNKE ET AL. and therefore shows the feasibility of optimizing commonly used labware with 3D-printing techniques and their comparision to conventional fabricated labware [1].
Going further from the specific results of this experiment, this study demonstrates the applicability of the novel manufactured printed labware. This enables the possibility to adapt the labware to the specific needs of the organism being used, regardless of their wide differences.

4. Concluding remarks

Our results demonstrate the possibility to integrate 3D-printed devices or culture vessels in a dedicated laboratory environment. It enables more time-efficient development of new prototypes and will help scientists around the world to create almost any construction without needing dedicated knowledge and skills of complex manufacturing processes. Precision engineering workshops are often not available for all laboratories or are not able to create complex working prototypes within a short time. Furthermore RP is very cost-effective if small quantities are needed which is often the case for prototypes or individual solutions. With this new technology it is basically sufficient to use CAD-software to create the desired geometries and subsequently transfer those to a 3D-printer.
Labware being used now was designed long ago and with only a few organism in mind, which had at that time not so complex requirements. More sensitive and demanding organisms, like mammalian cells, require more sophisticated conditions. Biocompatibility of this new labware must be certified and should be able to withstand the relatively long duration of mammalian cell cultivations. The experiments using extraction media and the two adherent cell strains A549 and NIH-3T3, as well as the suspension cell strain CHO-K1-HP, confirm the biocompatibility of the already certified PA 12-material, as well as the so far uncertified PA 12 UV-material. Furthermore the improvement of the UV-stable material by adding antioxidants benefit the proliferation conditions for the cell culture. This is reflected in better cell growth compared to using the USP-certified PA12-material.
The yeast cultivations in baffled well plates exemplifie the usability and possibilities of design driven 3D-printing in a biotechnological laboratory. It was possible to reach a sufficient oxygen intake by adding different baffle-geometries. More important than the results itself is that this experiment

shows how easily a new design for e.g. a well plate or any other cultivation vessel can be materialized with RP-technology.

One of the present problems with 3D-printing and especially SLS-technology concerning labware is the leak-tightness of the printed object. SLS is the most suitable 3D-printing method, because of the wide range of applicable materials, but the sintering-process itself has to be further optimized. The results exposed here, show that it is possible to create complex labware that is biocompatible and can be used in real life biotechnology applications. Also we could observe that the process of SLS-fabrication does not change the properties of the material with regard to the biocompatibility.

The future RP-technology will support new approaches in developing novel products for biotechnology purposes such as probes, reactors or basic consumables. As a result it will also have an effect on the logistics. A laboratory of the future may not need to rely any more on elaborate stock keeping of all necessary labware, it will be able to maintain its own inventory and produce new consumables over night or just in time on demand. Therefore even the direct integration of future generation 3D-printers in laboratory benches is imaginable.

The laying of the foundation stone for this future vision was the expiration of one major patent for selective laser sintering on January 28th, 2014 [14]. Many other key patents connected with 3D-printing are expiring from now until the middle of 2015 [15–25]. As patents expire novel printers become more affordable and the development and improvement of the techniques advance rapidly. Similar to the 3D-printing revolution that accompanied the expiration of major FDM patents a new movement is expected to appear. This second revolution will shake up the industry and make the yet high priced devices more affordable for small companies and especially laboratories. With the future advancements in premises to the printer technology it will be possible to use the rapid prototyping directly in a dedicated biotechnological laboratory for creating the needed labware or even complete bioreactors. This would lead to new approaches of developing and producing consumables and is, as a consequence, an important topic for today's manufactures to deal with.

Conflict of interest statement

The authors have declared no conflict of interest.

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Figure 1: Rendered CAD-image (A) and photo of the printed baffled well plate type "B" (B). The printed plate was used for cultivation experiments with *S. cerevisiae*. A 3D-view of the well plate type "B" is available in the online supporting information.

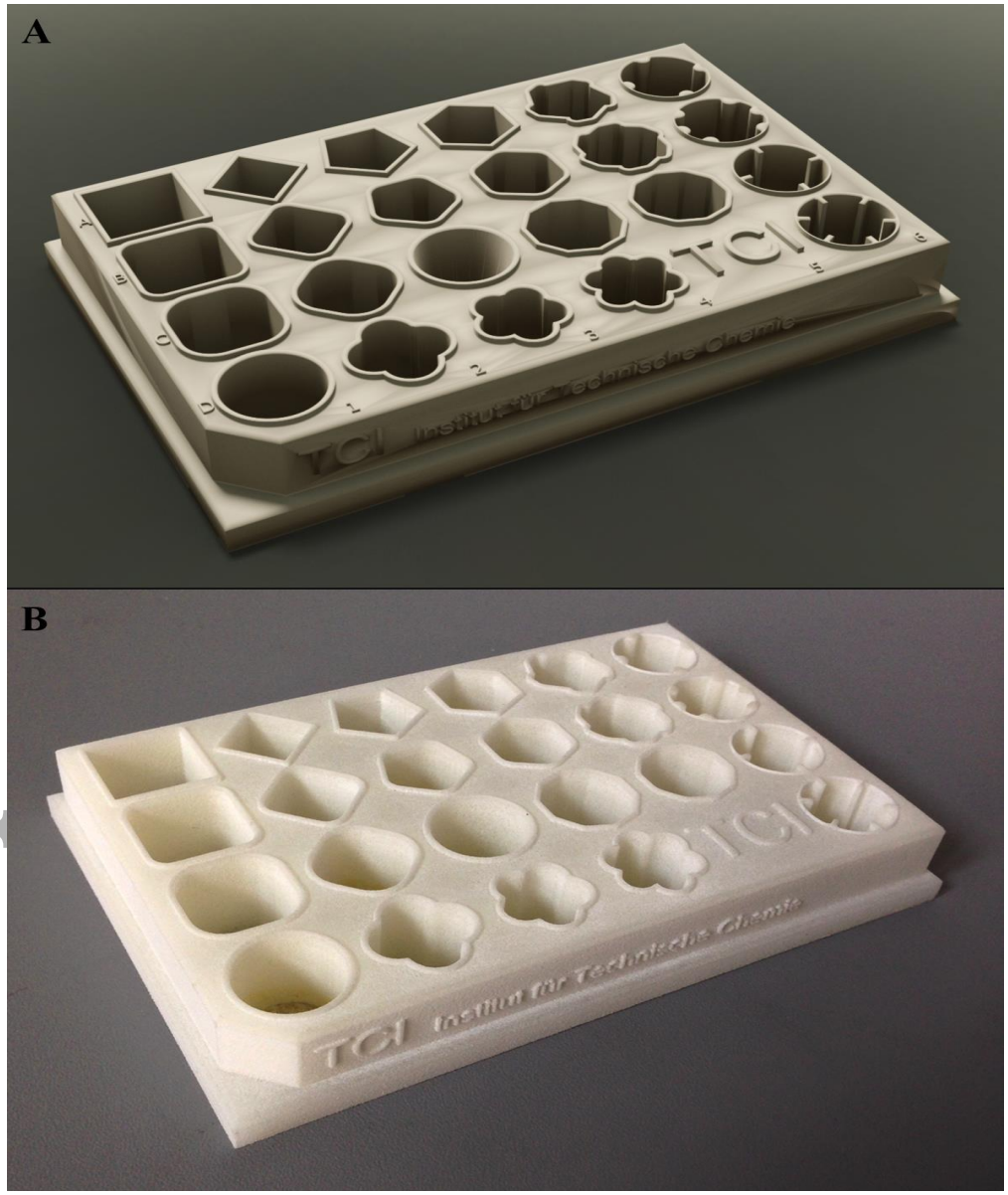

Figure 2: The relative resorufin concentration compared to the reference. Resorufin is reduced by viable cells and thus provides an information about the culture viability. The experiments were replicated thrice with four replicas within each experiment (error bars indicate the SD of the experiments).

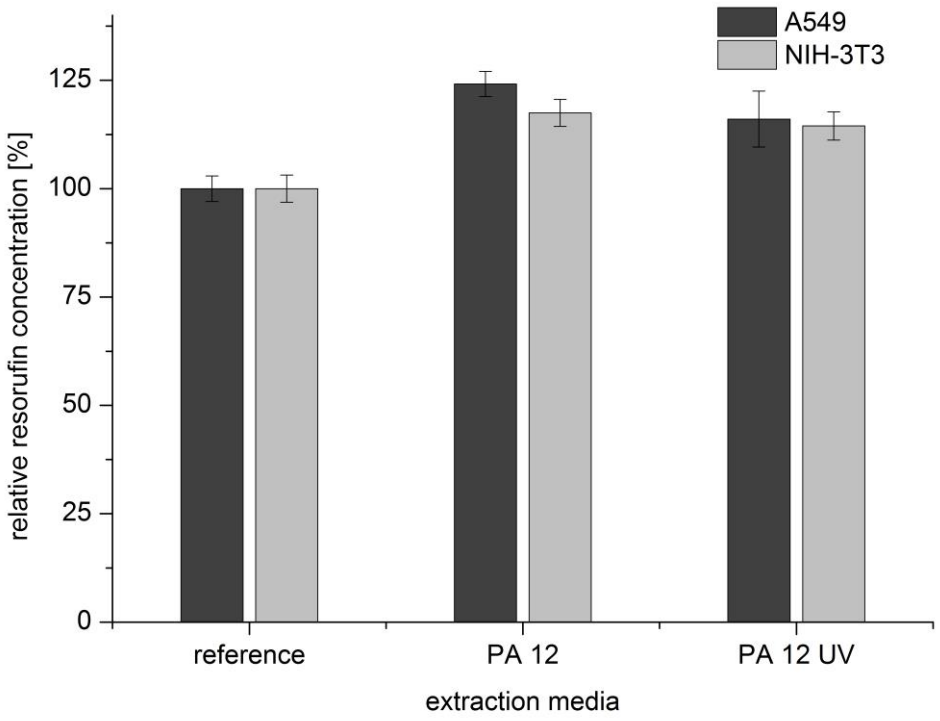

Figure 3: Absolute cell count per well (average of four wells, error bars indicate the SD of the experiments). Cells were cultivated for 48 h and detached using Trypsin. Counting was performed manually with a Neubauer chamber.

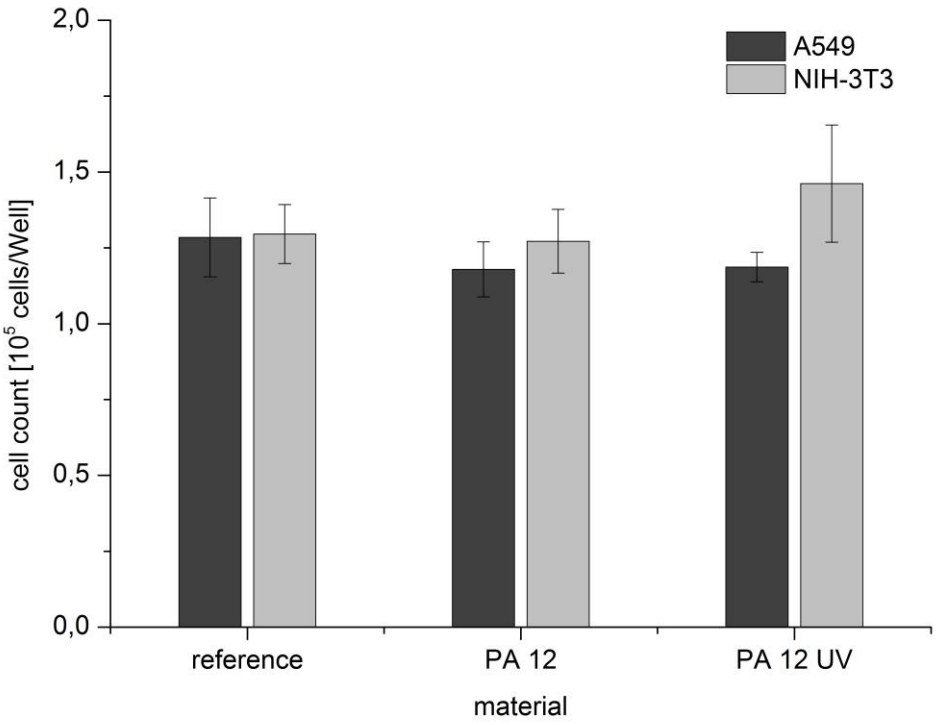

Figure 4: Relative LDH-concentration after lysis of the cells. Because of the cell lysis, these results provide information about the absolute cell concentration in each well. The shown data are an average of four parallel experiments (error bars indicate the SD of the experiments).

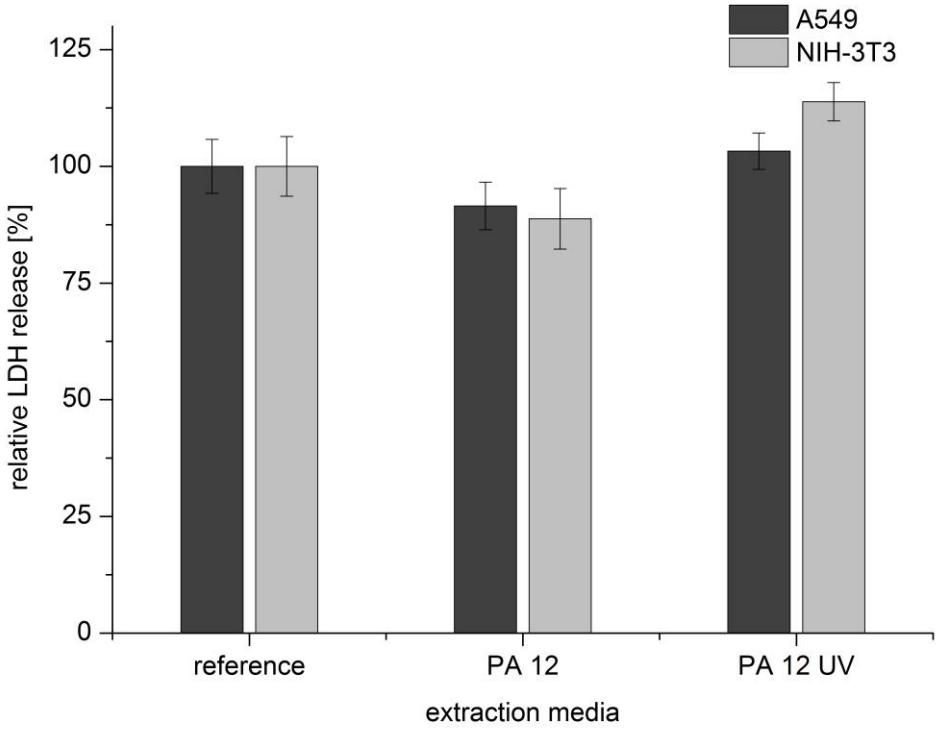

Figure 5: Relative LDH-release in the supernatant without cell lysis. These measurements are used for determination of cell death. The LDH-release in the supernatant is caused by the loss of membrane integrity of the cells and indicates necrotic cells. Both cell strains were cultivated in 3D-printed replicas of a 24 well plate, as well as in the standard well plate as a reference. The shown data are an average of four parallel experiments (error bars indicate the SD of the experiments).

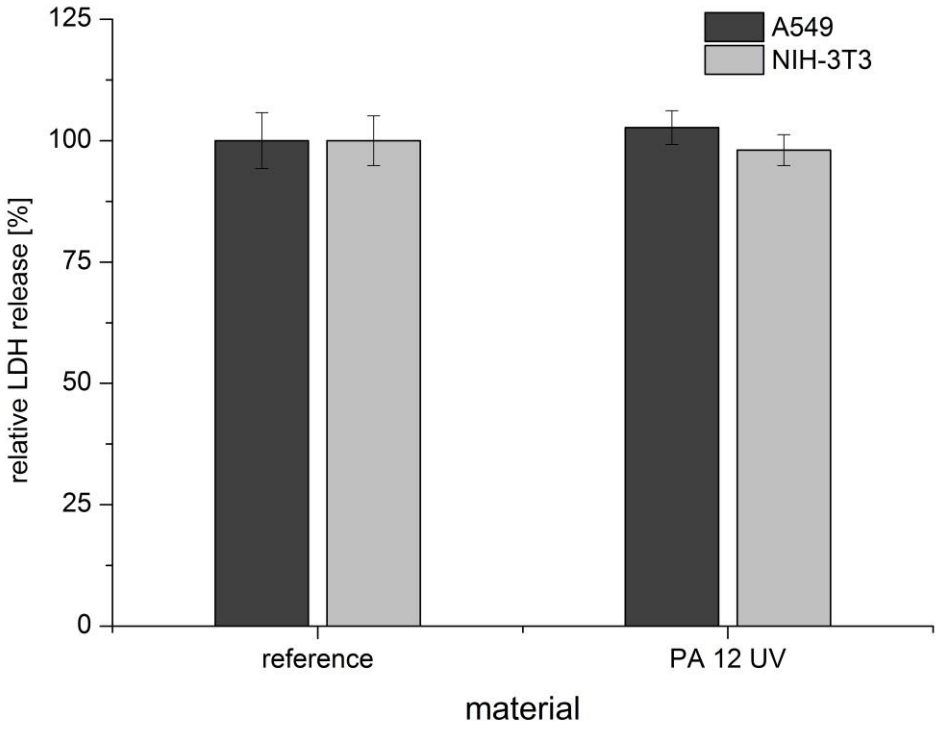

Figure 6: CHO-K1-HP growth curve, showing the viable cell concentration. The cells were cultured in CHOMACS CD media at 37 °C and 5 % (v/v) CO_2 at 160 rpm orbital shaking. The shown data are the average of two parallel experiments, error bars indicate the SD.

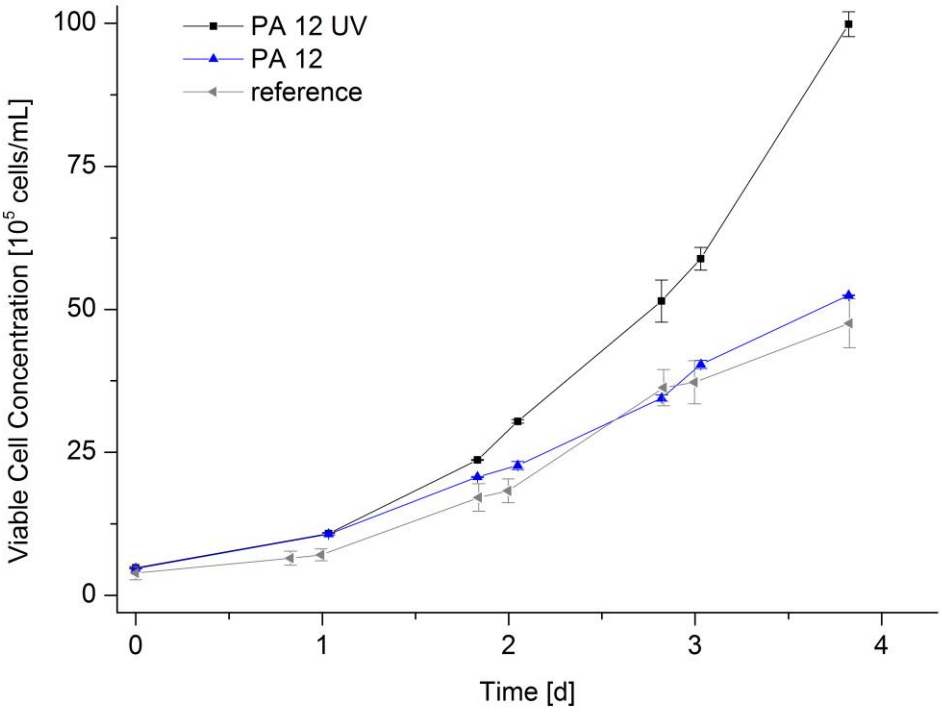

Figure 7: Results of *S. cerevisiae* cultivation in 3D-printed well plate type "B". Columns show the $OD_{600\,nm}$ measurements after 24 h of cultivation at 30 °C and 175 rpm. Shown here is the influence of increasing the corner radius from square until a completely round well. This experiment was repeated three times (error bars indicate the SD of the experiments).