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3D tissue culture substrates produced by microthermoforming of pre-processed polymer films

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Abstract We describe a new technology based on thermoforming as a microfabrication process. It significantly enhances the tailoring of polymers for three dimensional tissue engineering purposes since for the first time highly resolved surface and bulk modifications prior to a microstructuring process can be realised. In contrast to typical micro moulding techniques, the melting phase is avoided and thus allows the forming of pre-processed polymer films. The polymer is formed in a thermoelastic state without loss of material coherence. Therefore, previously generated modifications can be preserved. To prove the feasibility of our newly developed technique, so called SMART = Substrate Modification And Replication by Thermoforming, polymer films treated by various polymer modification methods, like UV-based patterned films, and films modified by the bombardment with energetic heavy ions, were post-processed by microthermoforming. The preservation of locally applied specific surface and bulk features was demonstrated e.g. by the selective adhesion of cells to patterned microcavity walls.

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1. Introduction

Organotypic cell culture systems have become indispensable in many applications where the three-dimensional organ environment of a cell influences the cell's behaviour to a great extent. This is true for basic research in modern cell biology, but also for applications in toxicology, cell oriented diagnostics as well as for cell and organ therapy. Therefore, advanced cell culture systems (i) should provide organoids to study drug metabolism and toxicity comparable to animal models, (ii) should allow the application of a limited supply of target cells like biopsy material, and finally, (iii) may also be up scaled to reach a long term viability and organotypic performance comparable to organs in vivo for emergency treatments of acute organ failures. Some important organotypic cell culture systems are described in Desai et al. (1999), Eschbach et al. (2005), Kunz-Schughart et al. (2004), Leclerc et al. (2003), Snyder and Desai (2001), Yamauchi et al. (2003).

The cellular microenvironment controls the viability, i.e. proliferation, differentiation and metabolism of cells (Semino et al., 2003; Takezawa, 2003; Yeung and Leckband, 1997). In case of anchorage dependent cells the main microenvironmental parameters are the topology of the substrate, the composition of the extracellular matrix, soluble factors and possibly their local concentration gradients and signals of neighbouring homo- or heterotypic cells.

Since most of the afore mentioned parameters can be controlled directly or indirectly by the design of the culture

substrate, tissue engineering in practice starts by substrate engineering.

Three dimensional tissue models unquestionably exhibit numerous advantages as compared to monolayer cell cultures and are important for *in vivo* or *ex vivo* medical applications (Tan and Desai, 2003). Up to now most 3D tissue scaffolds are of foam-like structure or microcapsules (Chia et al., 2000; Funatsu et al., 2001; Hasirci et al., 2001; Ranucci et al., 2000). Both techniques have inherent deficiencies with respect to fluidic properties necessary for vascularisation or controllable gradients of soluble factors and metabolic compounds. To overcome this we introduced earlier a microstructured array of microcontainers in polymer technique, which allow a three-dimensional culture or co-culture of cell lines as well as primary cells (Weibezahn et al., 1994). Housing in a containment forms a bioreactor which permits culture of complex tissue layers under perfusion and/or superfusion conditions.

As the production of these microstructures is very complex and expensive we developed a new cheaper and even more versatile technique. Here we present a novel approach to design three-dimensional polymeric tissue culture substrates in a chip format. Microcavities with a diameter and depth of 300 μm are arranged in an array with the dimension of 1 $\times$ 1 cm^2. For tissue engineering purposes, each microcavity is completely filled with cells, forming 3D cell aggregates of defined size and shape. The microstructured substrate offers a large surface area for cell attachment. By tailoring the surface properties of the microcavity insides, cell differentiation and viability can be affected and modulated as desired. Today, the large assortment of available polymers provides some benefits together with new challenges. A broad spectrum of polymers displaying different mechanical, chemical, optical and thermal bulk properties is available and can be modified regarding the surface properties of the device which link directly to the biological interface. Unfortunately, most surface and also most bulk modification methods are not compatible with typical moulding techniques.

In contrast to common micro moulding techniques, such as micro injection moulding, injection compression moulding and hot embossing, microthermoforming requires no steps within the polymers melting phase. So far, three dimensional polymer microparts and microstructured components can only be modified (e.g. adaptation of physicochemical properties of surface and bulk material or topology) after the moulding process due to the afore mentioned inherent melting phase. Here, the sequence of the processing steps is predetermined since surface modification techniques based on impinging beams (UV and X-ray lithography, particles for ion track technology) suffer from the hindered accessibility to inner surfaces of cavities. These limitations of tissue culture scaffolds are overcome by the presented microthermoforming of polymeric source materials.

Microthermoforming was recently adapted by us from the macroscale with all the benefits of the powerful macroscopic process, e.g. short cycle times and the possibility to operate the process in a batch or continuous mode (Truckenmüller et al., 2001). Starting from thin polymer films, microthermoforming typically results in thin-walled, hollow 3D structures. Adequate aspect ratios of such structures are about 1 and their size is usually in the range of a few 10 μm. Due to the very thin walls, microthermoformed parts are flexible but, nevertheless, are mechanically stable, so that there are no substantial problems concerning the handling or the demoulding of the parts. This technique seems therefore especially suitable for a high-yield production of chip-based microstructures for life-science applications.

2. Methods

2.1. Applied polymers and characterisation

The polymers used for microthermoforming and subsequently as cell culture substrates were polystyrene, PS (NSW, Norflex, gauge 50 μm, biaxially oriented) and different types of polycarbonate, PC (LoFo High Tech Film GmbH, Pokalon OG 461 Gl, gauge 50 μm, isotropic solvent cast film = Type 1; Pokalon N49 EM, gauge 40 μm, isotropic solvent cast film = Type 2).

The thermoplastic films were thermoanalytically characterised according to ISO 11357-3 by differential scanning calorimetry (NETZSCH DSC 204 Phoenix®) to determine the glass transition temperature. Three steps (1. heating, 2. cooling, 3. heating) were performed in the temperature range of 50°C to 150°C for polystyrene and 110°C–210°C for PC with a heat rate of 10 K/min.

2.2. Auxiliary patterns

An auxiliary pattern on the flat polymer film surface with silver spots of approximately 100 nm thickness was produced by a mask based sputtering process (Balzers, Med 010). For this, a nickel mesh with the outer dimension of 25 $\times$ 25 mm^2 and a mesh size of 25 $\times$ 25 μm^2 (Plano, G248N, 1000 nickel mesh) was placed on the polymer surface. Electrostatic forces were sufficient to ensure its adhesion to the polymer surface.

2.3. Photochemical surface patterning

The physicochemical surface modification of planar polymer films was performed by UV irradiation of the polymer samples in air using a low pressure mercury lamp (Heraeus Noblelight GmbH, Kleinostheim, Germany, NNQ lamp, quartz tube, 15 W) emitting λ = 185 and 253 nm at 10 cm distance with exposure times of 30 minutes. For patterned exposure a chromium quartz mask was placed in contact

to the polymer films. For details see (Welle and Gottwald, 2002).

2.4. Ion track technology

The modification of the bulk material by accelerated heavy ions was performed at the UNILAC linear accelerator of GSI (Darmstadt, Germany). PC films were irradiated perpendicular to their surface with xenon ions of 1460 MeV energy at a fluence of about 10^6 ions/cm^2. The ions have a penetration depth of approximately 150 μm in polymers and induce a permanent material modification (Fleischer et al., 1975; Spohr, 1990). The wet chemical etching process was performed by immersing the polymer parts for 6 h at 50°C in an aqueous solution of 5 Mol/L NaOH and 10% w/v methanol. The pore size was controlled by etching time and the temperature of the etchant. The etching process was stopped by rinsing the parts thoroughly with deionised water.

2.5. Microthermoforming

The microthermoforming process was performed on a regular hot embossing machine. The adapted tool consists of two circular brass plates, the negative mould and a counter plate with integrated vacuum and pressure ports. The mould is provided with a square array of 25 × 25 cylindrical microcavities with a diameter of 350 μm and a depth of 300 μm.

The thin thermoplastic film is clamped in between the two brass plates and then heated to a temperature near its glass transition temperature by contact heating. Subsequently, the entropy elastic film is stretched by a differential gas pressure into the preliminary evacuated micromoulds. To facilitate the demoulding step, the micromoulds are provided with drafts (5° inclination) and 45° chamfers at the upper edge. For details see (Giselbrecht et al., 2003, 2004; Truckenmüller et al., 2003).

2.6. Substrate finishing, cell culture and histology

UV treated and microthermoformed cell culture substrates can be conveniently sterilised with γ-radiation from a ^{60}Co source. Therefore samples were sealed in polyethylene bags and exposed to γ-radiation.

Prior to cell culture, the substrates were treated stepwise with decreasing concentrations of isopropanol to remove air bubbles entrapped in the microcavities.

Standard cell culture was performed in 250 mL tissue culture flasks (Becton Dickinson Labware, Plymouth, England, Falcon). Cells were maintained under culture conditions of 37°C, 100% relative humidity, 5% CO_2/95% air. We used the Human Caucasian hepatocyte carcinoma cell line Hep G2, ECACC Ref. No. 85011430, and murine fibroblast cell line (L929). Hep G2 cell culture medium was Minimum Essential Medium (MEM) supplemented with 1% w/v non-essential amino acids solution, 2 mM L-Glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin (all media components delivered by Invitrogen BV) and 10% w/v foetal calf serum (PAA Laboratories GmbH, Linz, Austria). L929 cell culture medium was MEM supplemented with 2 mM L-Glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin and 5% w/v foetal calf serum.

Cell suspensions were prepared using 0,2 w/w trypsin (Life technologies) in a solution of 4,5 g/L sodium citrate (Na_3-citrate $2H_2O$), 10 g/L KCl, 3,85 g/L EDTA and 5 mg/L phenol red to detach cells from the culture substrate after rinsing the culture flask with Ca/Mg free phosphate buffered saline. Cell counting was performed with a hemocytometer.

For the cultivation of cells (L929, Hep G2) on UV patterned polymers, the medium was supplemented with 1% w/v Pluronic F68 solution (Sigma, Munich, Germany) (Dewez et al., 1998).

For cell fixation and staining, cellularised microstructured polymer substrates were rinsed with phosphate buffered saline (PBS) to remove medium by washing before they were totally immersed into the crystal violet solution (5,0 g/L in methanol, Serva Feinbiochemika, Heidelberg, Germany) for approximately 30 min. Afterwards, the tissue culture substrates were carefully immersed in deionised water several times and air-dried.

3. Results

3.1. Characterisation of polymeric materials

The polymer processing temperature is one of the key parameters of microthermoforming. Most polymers are featuring good formability only within a narrow temperature range of a few Kelvins e.g. near their glass transition temperature. Therefore, a preliminary examination of the glass transition temperature of the used amorphous polymers, polycarbonate PC and polystyrene PS, with differential scanning calorimetry (DSC) was performed. The glass transition temperatures of the two mentioned thermoplastic polymers were determined according to ISO 11357-3 with a heat rate of 10 K/min for PC to 159°C and for PS to 101°C.

3.2. Durability and distortion of surface features

To detect distortions of the surface modification patterns, which are expected to be caused by the thermoforming process, we introduced prior to the forming step a silver spot pattern on PC films (Type 1). Furthermore, thin metal coatings could also be used as starting layers for self-assembled monolayers, SAMs, e.g. to control cell adhesion (Chen et al., 1998; Martelé et al., 2003; Mrksich and Whitesides, 1996).

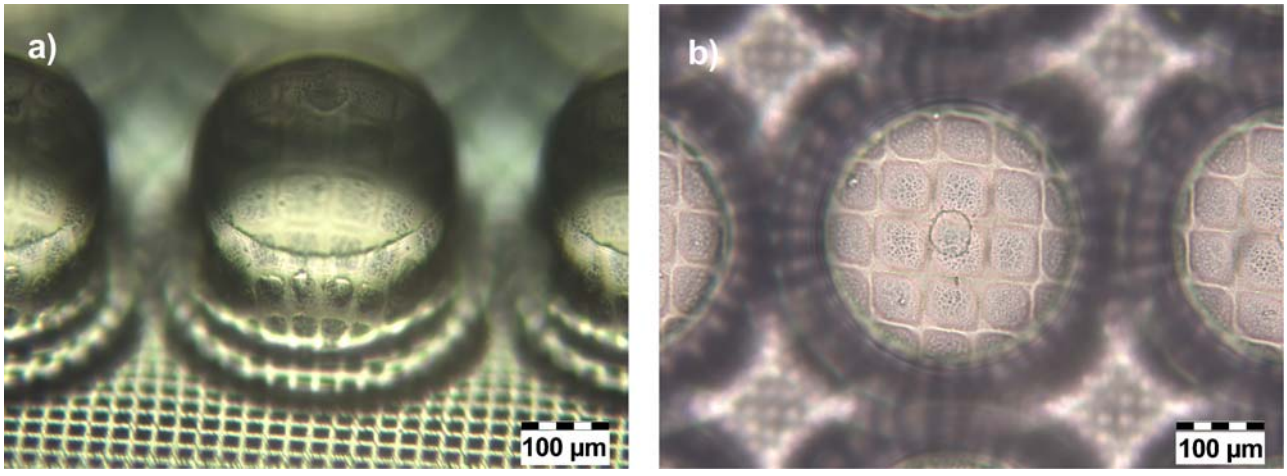

Fig. 1 Microthermoforming of PC films patterned with thin silver spots. The unstretched region in the foreground of the microcavities in the left picture (a) shows the original pattern of the 25 × 25 μm^2 sized silver spots

The mask based sputtering process with silver onto the plane films lead to a pattern of 25 × 25 μm^2 sized quadratic spots with a thickness of about 100 nm. The subsequent microthermoforming of this patterned film with a pressure of more than 4000 kPa resulted in microcavities having a width of 350 μm and a depth of 300 μm and a maximum draw ratio at the top of about 4. In comparison, there was no observable difference in the processability of a patterned or non-patterned PC film. Fig. 1 (a) and (b) show the original pattern of silver spots and the stretched pattern on the inside of the microcavity walls as well. The size of the spots corresponds directly to the local stretching factor, which increases non-linearly from the base to the top. Therefore, the distortion of the original pattern is predictable if the distribution of the local draw ratios is known. Especially the high stretching of the polymer film at the top of the microcavities lead to a tearing of the original compact silver spots.

3.3. Microthermoforming of polymers with random surface topology

Besides surface coatings, the microthermoforming process enables also the 3D forming of films with a surface roughness or a defined topology. Textured surfaces and certain topologies inside microcavities of cell culture substrates can be very important as it could be shown that they can promote three-dimensional tissue formation (Knedlitschek et al., 1999). To prove the preservation of surface structures, we used a one-sided stochastically textured and commercially available PC film (Type 2) (see Fig. 2(a)). The polymer underwent the same moulding procedure as described in part 3.2. The depth of the microcavities after microthermoforming was approximately 260 μm. So far, the original roughness of the film and the roughness inside the formed microcavities were not measured, but according to our other experiments,

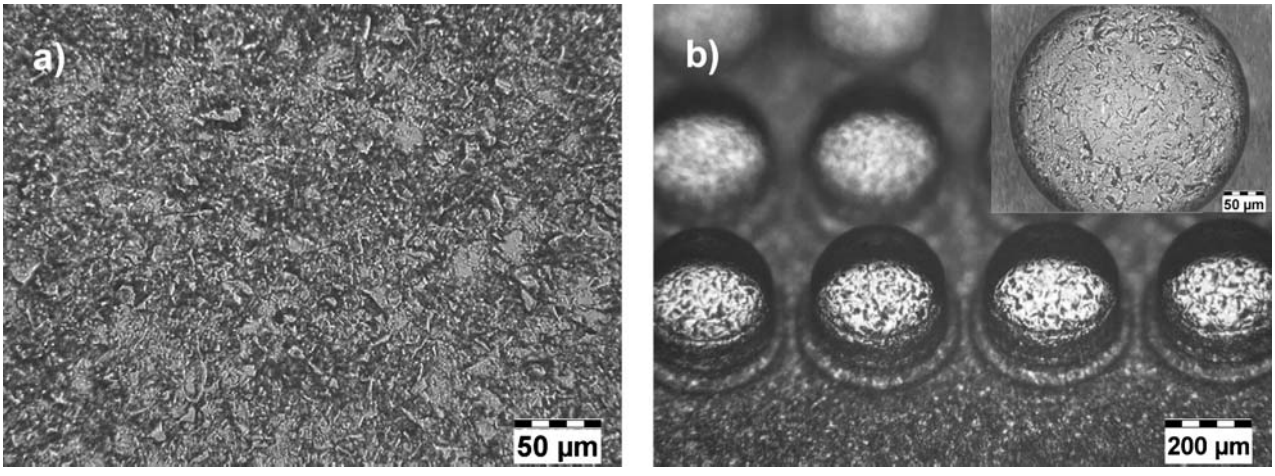

Fig. 2 SMART processed thin polymer film with a stochastic surface topology (before (a) and after (b) the microthermoforming process; inserted picture shows the surface roughness on the inner side of a single microcavity from the reverse, microcavity depth ≈ 260 μm, scale bar 50 μm). Inserted picture on the right was digitally remastered with analySIS (Soft Imaging System GmbH) by extended focus imaging

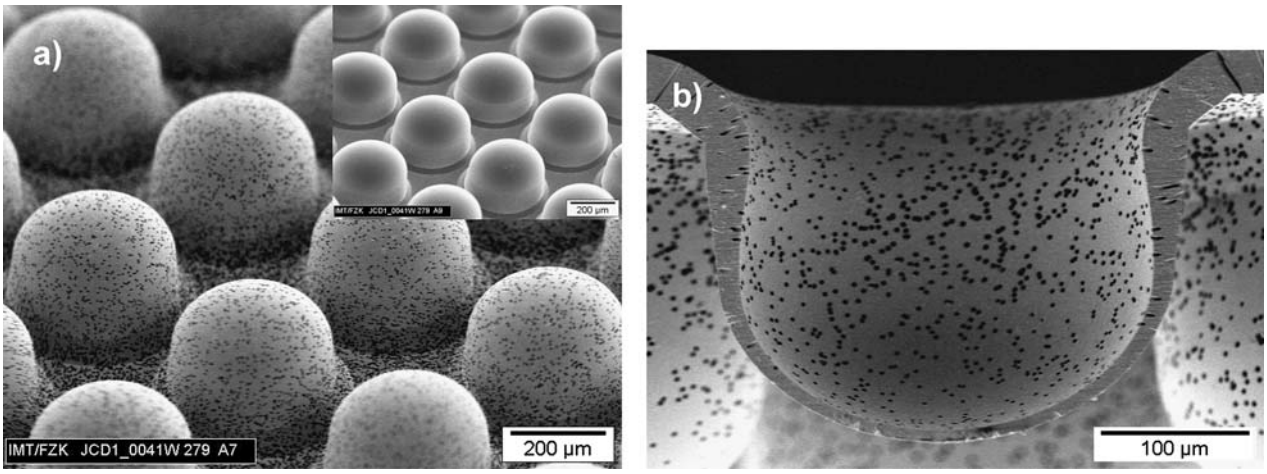

Fig. 3 Ion track technology combined with microthermoforming for the fabrication of highly porous, three dimensional microstructures. (a): PC films irradiated with Xe ions (1460 MeV, fluence 10^6 ions/cm^2) and microthermoformed before (inset, scale bar 200 μm) and after the etching process for 6 h at 50°C in 5 Mol/L NaOH/10% w/v MeOH. Note, that non-etched latent tracks are not visible by scanning electron microscopy. On the right (b): etched cross section of a single cavity

the topology of the roughness seems to be distorted proportionally to the local stretching of the film as can be seen in the light microscopy pictures of Fig. 2.

3.4. Highly porous tissue culture substrates produced by bulk modification and microthermoforming

In addition to the afore mentioned combinations of coatings and topologies with microthermoforming, this low temperature forming process can also be used to transfer polymer films into three dimensional structures that have localised material modifications induced e.g. by energetic heavy ions. The ion tracks are produced by exposing the polymer film to the beam of MeV-GeV ions, which penetrate through the bulk creating trails. From the pre-irradiated film, microcavities are fabricated by microthermoforming and finally the ion tracks are developed into cylindrical pores by chemical etching. As an example, PC films (Type 1) were first irradiated with heavy xenon ions of 1460 MeV energy at the UNILAC linear accelerator of GSI (Darmstadt, Germany). During the subsequent microthermoforming process, the cohesion of the polymer film was maintained even in the modified regions, so that the formability of the film was comparable to untreated PC films (see Fig. 3(a)). The depth of the obtained thin walled hollow microcavities was approximately 250 μm and the width was again 350 μm. In a first set of experiments even depths of 300 μm were achieved without any tearing of the polymer film.

Subsequently, pores were produced by wet chemical etching of the thermoformed microstructures with 5 Mol/L NaOH/10% w/v MeOH. In principal the pore density in the unstretched material can be controlled via the ion fluence within small limits (each ion produces an etchable track) and the pore size by the etching conditions (etch time, concentration etc.). Stretching after irradiation results in higher local variation of these parameters. An increase of the local stretching factor is associated with a decrease in local pore density and an increase in pore size. The pore size of one specimen etched for 2 h at 40°C e.g. varied from approximately 400 nm in the unstretched regions to a size of approximately 1600 nm at the top of the microcavities. In certain applications these variations have to be taken into account.

One of the major advantages of the applied process sequence is shown in the scanning electron micrograph of the cross section of Fig. 3(b): Every part of the microcavity walls, side walls included, can be provided with micro- or nanopores. Therefore, the SMART technology enables the production of microcavities with an increased number of pores per microcavity at a relatively constant pore density. Thus, cells immobilized inside these three dimensionally shaped, filter-like microstructures can be supplied with nutrients and gases from all sides. The patency of the pores was proven by perfusion of bioreactor mounted microstructured tissue culture substrate with coloured water.

Depending on the angle of incidence of the energetic ions on the flat polymer film, the pores can be oriented as desired to the microcavity surface. Moreover, if the flat polymer film is irradiated through a mask, designated areas, e.g. the areas between the microcavities, can be omitted from the perforation process. As a consequence, the locally modified polymer film has then to be aligned with the microthermoforming tool.

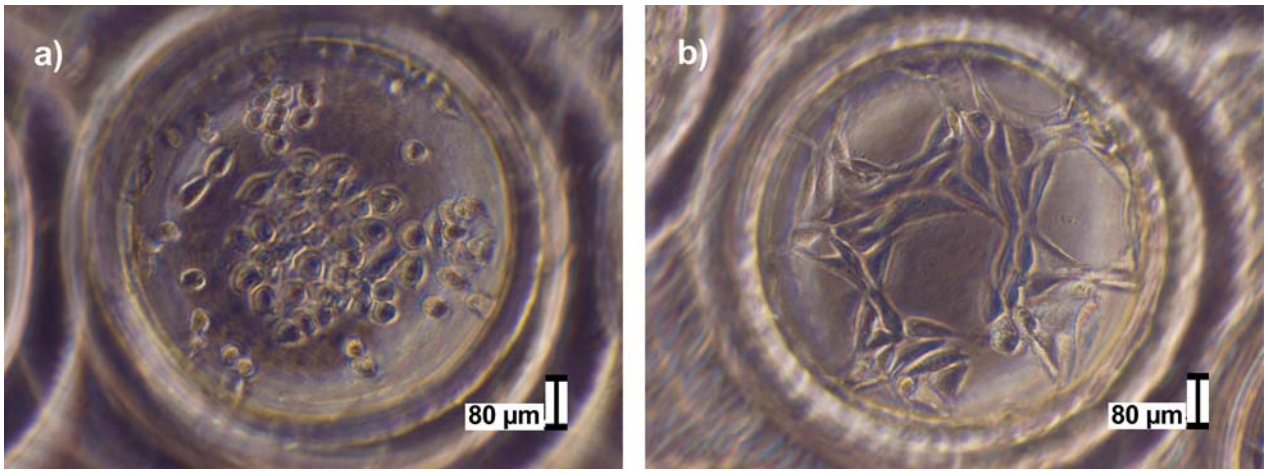

Fig. 4 SMART processed scaffold with a three dimensional cell adhesion pattern. Living fibroblasts in microcavities without (a) and with (b) a patterned surface (PS, day 3 of cultivation). Phase contrast microscopy from the rear side

3.5. Tissue culture substrates produced by UV-patterning and microthermoforming

For the envisaged application as a tissue culture system, the feasibility of microthermoforming in combination with physicochemical surface cell patterning methods, here a UV-irradiation based method, was examined. In general appropriate adhesion patterns of subcellular dimension on the inner side of the microcavities can be applied to promote a three dimensional cell growth (A. Welle, personal communication). By this the well known contact inhibition of confluent monolayer cells can be prevented, which would otherwise occur if only a small number of proliferating cells is inoculated into the cavity.

In the following experiment we used not a subcellular patterning but rather a more macroscopic pattern to get a visual proof for the pattern stability after microthermoforming. Therefore, a PS film was photochemically patterned by a 30 min. UV-irradiation through a chromium quartz mask that consisted of a checkerboard pattern with the size of $50 \times 50\,\mu m^2$ of each square. Subsequently, the patterned film was microthermoformed to an array of 25×25 microcavities with the same dimensions as in the previous experiment (350 μm width, 300 μm depth). Additionally, non-irradiated microthermoformed PS cavities were used as a reference substratum for cell culture.

In order to prove the preservation of the local surface activation and to be able to observe a spatially differentiated cell adhesion, the microcavities were not completely filled with cells, but only a small number of fibroblasts (L929) were inoculated into the microcavities and cultivated for eight days. Already two hours after inoculation, the adhesion of the cells to the patterned microthermoformed surfaces compared to the non-patterned reference structures was clearly distinguishable. Figures 4 and 5 show the selective cell adhesion on day 3 of cultivation. Cell adhesion was

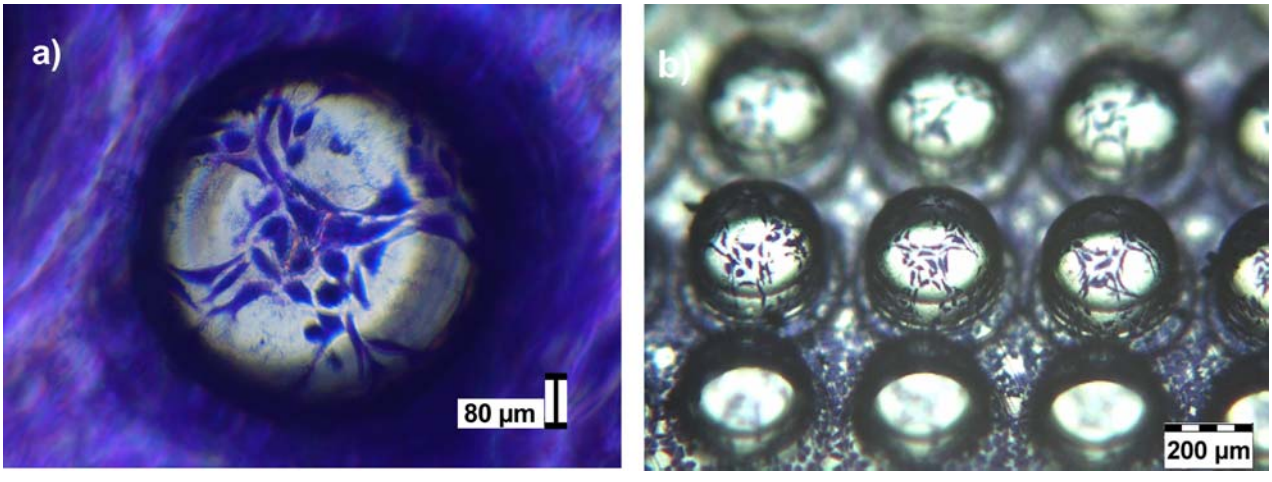

Fig. 5 Fixed and crystal violet stained fibroblasts (L929) inside UV-patterned microcavities (day 3 of cultivation). The figure on the left (a) shows a single microcavity from the back. On the right (b), several microcavities are displayed from a different angle

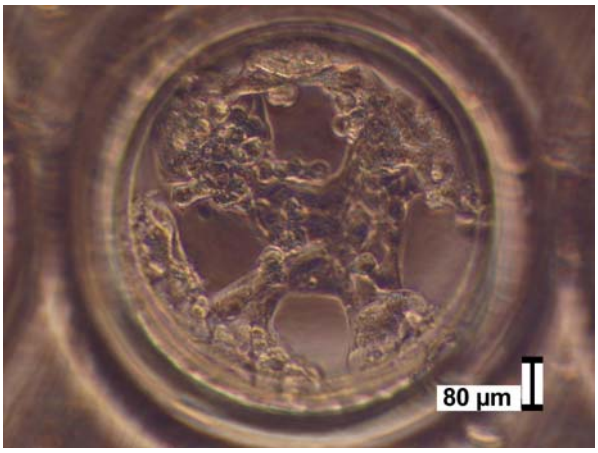

Fig. 6 Hep G2 cells in photochemically patterned microcavity made from PS (day 2 of cultivation). Same pattern as above. Phase contrast microscopy

restricted to the irradiated areas of the pattern, whereas the non-irradiated spots were avoided. Fibroblasts on the non-irradiated polystyrene of the reference microstructures did not adhere firmly to the surface. As a result, they assumed round shaped cell bodies, typically observed on untreated standard bacteria culture dishes produced from native polystyrene. These observations are in accordance with our results of previous investigations on two dimensional patterned PS substrates (Welle, 2003; Welle et al., 2002).

The arrangement of the fibroblasts on the partially UV-modified microstructures represented the distorted pattern fairly well. As in the case of the inorganic pattern of the silver spots, the size of the "cell spots" at the top of the microcavities (approx. $100 \times 100\ \mu m^2$) again reveal the maximum draw ratio of about 4.

The encouraging results achieved with L929 fibroblasts were reproduced with a second cell line, the human hepatoblastoma line Hep G2, which is more demanding concerning the surface properties of the adhesion substrate. Even with these cells the pattern could be reproduced, but the non-irradiated and irradiated regions were not as well-defined as

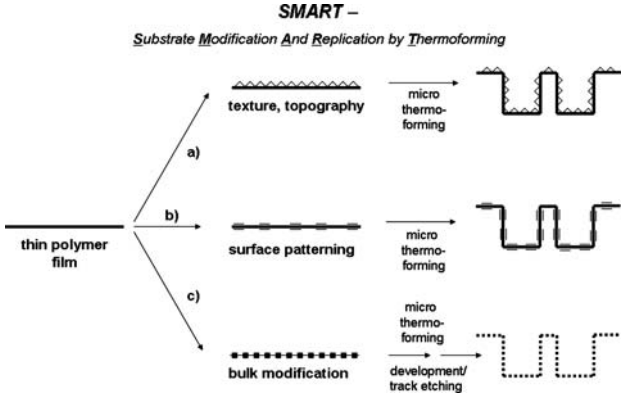

Fig. 7 Microthermoforming of pre-processed, modified polymer films (textured, surface and bulk patterned)

in the case of the fibroblasts (see Fig. 6). This is most likely due to differences in the morphology and plasticity of both cell types.

4. Discussion

4.1. Principle characteristics of the SMART process

So far, available technologies for the production of microstructured tissue engineering scaffolds that allow for the generation of spatially determined surface and bulk properties in addition to defined geometries and substrate polymers, have been very restricted. This arises mainly from the fact that most established methods of microstructure technologies like moulding (and most rapid prototyping techniques) are based on liquid or powder phase processing (melted, dissolved, sintered) of polymers (Heckele and Schomburg, 2004), which require a pre-assigned sequence of processing steps, where the surface and bulk modifications are located downstream in the process chain. However, it is nearly impossible for almost all modification processes to get access to the complete surface or the bulk material inside buried, narrow cavities or inside buried, long covered microchannels, especially if a patterned modification is needed.

The newly developed technique, SMART, gives rise to a broad spectrum of applications requiring the possibility of various surface and bulk modifications. Due to the comparative low forming temperature during replication by microthermoforming this method allows for a preferable reorganisation of the process steps within the whole sequence (Fig. 7).

As depicted in Fig. 7(a) microthermoforming can be readily combined with embossing/nanoimprinting, milling, (laser-) ablation, sand or powder blast processes etc. to create textured surfaces or a defined surface roughness. In extreme cases, distinct topologies could also result in thin walled hollow bodies with real undercuts on the side walls. Nanometer sized patterns, so far only possible in two dimensions due to the need of a contact mask, are now amenable to the third dimension by post-processing with microthermoforming. Moreover, this new microstructure technique can be combined with modification processes dependent on direct mask or stamp contact to the substrate for surface patterning (e.g. by UV-patterning, microcontact printing) as depicted in Fig. 7(b) or processes based on ionising irradiation (e.g. with X-rays, accelerated heavy ions) for local bulk material modifications, as depicted in Fig. 7(c). The adapted sequence of the processing steps enables the production of highly porous microcavities with pores oriented perpendicular to the bottom and side walls.

Up to now, only some examples of the huge variety of polymer processing methods are depicted in Fig. 7 and were tested

in our laboratories. Further existing polymer modification procedures (SAMs, plasma processes, printable local coatings, nanoimprint, soft lithography, ...), which so far have been limited to two dimensional substrates, should also be combinable with microthermoforming.

In addition to the afore mentioned pre-thermoforming steps some post-thermoforming treatments with fluids and gases or plasma based methods (inert and reactive gases, plasma enhanced chemical vapour deposition, etc.) are certainly applicable. In particular process combinations composed of lithography-based UV-activation, microthermoforming and grafting of bioactive molecules to these preserved activated sites in a finishing isotropic wet chemical coupling step should offer considerable benefits. Due to the possibility to expose the initially flat polymer substrate through a contact mask (no proximity effect!) not only patterns but also substrate-bound gradients of bioactive molecules should be producible with high resolutions.

Moreover, it should be mentioned that it is possible to apply the surface modifying techniques not only to one side of the film as depicted in Fig. 7(a) and 7(b), but also to the second side. This also offers the possibility to produce scaffolds which provide simultaneously areas for the three-dimensional and the two-dimensional culture of even different cell types. These different cells can also come in contact with each other over the pores of the film.

4.2. Points to consider

It was demonstrated that the special processing sequence of microthermoforming in combination with the techniques shown in Fig. 7(a)–(c) simplified the production of complex three dimensional polymer structures.

However, the alterations of the molecular structure and topology of the patterned and textured areas caused by the stretching process, e.g. near-surface rearrangement of functionalised polymer chains, cracks in coating layers, rounding of sharp edges, changes in pore size and shape etc., are not yet examined in detail. If necessary, the local stretching of the polymer film during the process has to be numerically simulated and the original pattern of the polymer film has to be adequately adapted. Moreover, possible alignment steps within the process sequence, e.g. by means of positionable thermoforming tools, could allow for a defined pattern of surface or bulk modifications on specified regions of the microthermoformed part. This also applies for the production of a patterned perforation by a mask-based irradiation of the plain polymer film with energetic ions e.g. in order to perforate the microcavities exclusively.

One of the major prerequisites for a successful processing of modified polymers is the need for a stable modification under the forming conditions applied. In case of polymers with relatively high forming temperatures, some temperature sensitive organic coatings will not tolerate these forming conditions, e.g. organic coatings like SAM's, which are frequently used in nanobiotechnology applications. If this problem cannot be circumvented by the use of polymers with forming temperatures near room temperature, such as polycaprolactone, microthermoforming offers another solution for this purpose. For this, the polymer film can be patterned prior to the microstructuring process with an inorganic coating (gold, silver etc.). The patterned inorganic layer could then act as a priming layer after the forming step for an isotropic coupling or grafting process of the organic coating on the pre-defined inorganic spots and areas.

Finally, the areal density of functional groups and deposited dose, respectively, inside stretched regions must not fall below a certain level to guarantee a sufficient contrast (concerning the application, such as cell adhesion) between pristine and modified material. For this, the geometry of the microstructure and the local stretching factor has to be adjusted to get an adequate contrast that is e.g. still recognisable for cells.

The SMART technology described here is a promising novel method to produce thin walled, non-buried, flexible and hollow microstructures that are intended to be subjected to additional modification technologies for highly specialised applications. Furthermore, it has to be noted that the central process of SMART, microthermoforming, potentially offers very short cycle times and can be performed in a batch or continuous mode. The process is therefore amenable to mass production.

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