Video Article

Microfabrication of Chip-sized Scaffolds for Three-dimensional Cell cultivation

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Abstract

Using microfabrication technologies is a prerequisite to create scaffolds of reproducible geometry and constant quality for three-dimensional cell cultivation. These technologies offer a wide spectrum of advantages not only for manufacturing but also for different applications. The size and shape of formed cell clusters can be influenced by the exact and reproducible architecture of the microfabricated scaffold and, therefore, the diffusion path length of nutrients and gases can be controlled.¹ This is unquestionably a useful tool to prevent apoptosis and necrosis of cells due to an insufficient nutrient and gas supply or removal of cellular metabolites.

Our polymer chip, called CellChip, has the outer dimensions of 2 x 2 cm with a central microstructured area. This area is subdivided into an array of up to 1156 microcontainers with a typical dimension of 300 µm edge length for the cubic design (cp- or cf-chip) or of 300 µm diameter and depth for the round design (r-chip).²

So far, hot embossing or micro injection moulding (in combination with subsequent laborious machining of the parts) was used for the fabrication of the microstructured chips. Basically, micro injection moulding is one of the only polymer based replication techniques that, up to now, is capable for mass production of polymer microstructures.³ However, both techniques have certain unwanted limitations due to the processing of a viscous polymer melt with the generation of very thin walls or integrated through holes. In case of the CellChip, thin bottom layers are necessary to perforate the polymer and provide small pores of defined size to supply cells with culture medium e.g. by microfluidic perfusion of the containers.

In order to overcome these limitations and to reduce the manufacturing costs we have developed a new microtechnical approach on the basis of a down-scaled thermoforming process. For the manufacturing of highly porous and thin walled polymer chips, we use a combination of heavy ion irradiation, microthermoforming and track etching. In this so called "SMART" process (Substrate Modification And Replication by Thermoforming) thin polymer films are irradiated with energetic heavy projectiles of several hundred MeV introducing so-called "latent tracks". Subsequently, the film in a rubber elastic state is formed into three dimensional parts without modifying or annealing the tracks. After the forming process, selective chemical etching finally converts the tracks into cylindrical pores of adjustable diameter.

Protocol

Process Sequence #1: Hot Embossing, Machining and Solvent Vapour Welding

The CellChip in its cubic design is replicated by hot embossing or micro injection moulding. For this, we use a micromachined brass mould with the inverse geometry of the chip. The containers - arranged in a regular array of up to 1156 containers - have a cubic design with an edge length of 300 µm. For hot embossing, the replication process is performed on a conventional WUM02 (Jenoptik Mikrotechnik, Germany). The tool consists of two circular metal plates. In a first step, a thin PMMA plate (Lucryl, G77Q11, BASF) is placed in the centre of the lower plate of the opened tool. The microstructured mould insert is centrally mounted in the upper plate. Then the tool is closed for evacuation of the mould and heated to a temperature above the glass transition temperature of the polymer. By pressing the plates together, the viscous polymer is pushed into the evacuated cavities until they are completely filled precisely replicating the geometry of the mould. After cooling the tool, the microstructured polymer part can be demoulded. The process requires more polymer mass than is actually needed to fill the mould cavities. The polymer surplus forms a residual layer which can be used to ease the demoulding of the part. However, to create pores of diameter smaller than 3 µm in the bottom of the containers, the thick residual layer has to be thinned down, or even completely removed and replaced by a porous membrane. To simplify the process of pore integration, the back of the replicated CellChip is completely removed by machining with a diamond mill. For this, the parts are fixed on a cooled mounting plate and, additionally, the fragile structures are frozen in deionised water to protect them against damage.

In a last process step, finally a commercial ion track-etched membrane (polycarbonate, thickness 10 µm, pore size 3 µm, 2x10⁶ pores/cm², Pieper Filter GmbH) is bonded to the back of the array of containers now opened both on top and bottom. The bonding process is a solvent vapour welding process performed in a gastight, heated chamber [Fig. 1], consisting of an upper plunger and a moveable lower plate with an integrated vacuum chuck.⁴ Up to four machined CellChips and track-etched membranes are exposed in parallel to a vaporized solvent after the chamber was evacuated. Then, the moulded parts and the membranes are pressed together by the upper plunger. After a short period of exposure (<15 s), the chamber is evacuated again thereby removing the solvent. Due to the short contact time, only surface near material is dissolved and a deformation of the bulk structure due to the mechanical load can be avoided. Finally, the chamber is opened and the solvent welded CellChips can be removed and prepared for cell culture [Fig. 2].

Figure 1.

Figure 2.

Process Sequence #2: heavy ion irradiation, microthermoforming and track etching (SMART process)

The new process called SMART is a recently developed micro technology for manufacturing functionalized membrane-like microstructures.⁵ The technology is based on a microtechnical thermoforming process, called 'microthermoforming'.^{6,7} In this central process step, which was adapted from the macroscopic trapped sheet thermoforming process, a heated thin polymer film is formed in its softened, rubber elastic state by gas pressure into a mould cavity [Fig. 3]. Unlike hot embossing or injection moulding, this process is not a primary forming and the polymer is not melted. Due to the fact that the film is formed still in a solid state with a permanent material cohesion, material modifications with high lateral resolutions can first be generated on planar polymer films and are preserved throughout the forming process. After the microthermoforming step, these modifications can be further selectively processed, e.g., by wet chemical treatment.

Figure 3

The SMART process in principle consists of three process steps:

1. creation of highly resolved modification patterns on planar thin polymer films in a pre-process
2. 3D shaping of films by microthermoforming without loss of (patterned) modifications
3. post-process (optional) for a final functionalisation of thin-walled microstructured parts

The SMART process we are currently applying for the fabrication of porous CellChips includes the following process steps [Fig. 4]. A thin polymer film, e.g., from polycarbonate (Pekalon OG461GI, 50 μm , LoFo High Tech Film GmbH, Germany), is irradiated with accelerated heavy ions (such as Xe, Au or U ions) at the accelerator facilities of GSI (Darmstadt, Germany) with energies of approx. 1 GeV and fluences in the order of 10^{18} ions/ cm^2 . When penetrating through the film, each ion produces a nearly straight trail of modified material, called latent track. The pre-treated films are then thermoformed to an array of 25x25 thin walled microcontainers, each with a diameter and depth of 300 μm . The process is currently performed on a modified hot embossing press [Fig. 5], where the polymer film is clamped in between two metal plates. The upper plate is equipped with the micromachined mould and the lower one contains the pressure and vacuum connectors. The film is stretched into previously evacuated microcavities of the mould by nitrogen with a pressure of up to 5 MPa. The films are formed near their glass transition temperature preventing track annealing.

Figure 4

Figure 5

In a post-process, the ion tracks are selectively etched to pores by immersing the entire microstructure into an appropriate etching medium (e.g., 5 Mol/L NaOH, 10% w/v MeOH). By controlling the etching times and etching conditions, such as concentration, temperature and special additives (e.g., etch promoters), the size and shape of the resulting pores can be adjusted [Fig. 6].

Figure 6

Discussion

Although established methods of polymer microreplication, such as micro injection moulding or hot embossing, are suitable for producing microstructures, they are not really effective in producing microstructures with an integrated and highly controlled porosity, as is needed for the CellChip. Bulky structures e.g. require costly machining to reduce wall thickness for a subsequent laser perforation or walls have to be completely

substituted by track-etched membranes. SMART is a new and promising technology that can overcome these problems and is suitable for mass production. Perspectives include manufacturing of thin-walled microstructures by roll fed, similar to production lines for macroscopic parts. Furthermore, the shaping of polymer films in a rubber elastic state offers the chance not only to create defined pores all over the structure (including vertical side walls) but also to provide microstructures with a highly resolved functionalisation, such as bioactive surface patterns, coatings, and topologies even inside hardly accessible, e.g., microfluidic cavities.

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