Kerstin Frey, Alena Fischer, Rumen Krastev and Ralf Kemkemer*

CurvChip - chip platform for investigating cell responses to curved surface features

Abstract: Surface topographies are often discussed as an important parameter influencing basic cell behavior. Whereas most *in-vitro* studies deal with microstructures with sharp edges, smooth, curved microscale topographies might be more relevant concerning *in-vivo* situations. Addressing the lack of highly defined surfaces with varying curvature, we present a topography chip system with 3D curved features of varying spacing, curvature radii as well as varying overall dimensions of curved surfaces. The CurvChip is produced by low-cost photolithography with thermal reflow, subsequent (repetitive) PDMS molding and hot embossing. The platform facilitates the systematic *in-vitro* investigation of the impact of substrate curvature on cell types like epithelial, endothelial, smooth muscle cells, or stem cells. Such investigations will not only help to further understand the mechanism of curvature sensation but may also contribute to optimize cell-material interactions in the field of regenerative medicine.

Keywords: photolithography, thermal reflow, topography with defined 3D curvature, chip platform.

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1 Introduction

Interactions between cells and their surrounding microenvironment have been extensively studied. Therefore, it is widely known that besides chemical factors of the extracellular matrix (ECM) its mechanical and geometrical cues influence cellular behavior[1,2]. In combination, ECM signals can have a decisive role in processes such as cell spreading, migration[3] or even stem cell differentiation[4]. Due to its importance for biomedical research and the development of improved implant surfaces, topography might be the most extensively studied parameter influencing cells. Well known, for example, is the alignment along elongated surface features leading to cell orientation or directed migration, also known as contact guidance or alignotaxis. However most in vitro studies focus on surface structures with sharp edges, often groove-like features, whereas smoothly curved topographies might be more relevant in mimicking the native cellular environment, since they are omnipresent *in situ*. These native curvatures vary from approximately 1 500 µm radii for the vena cava down to 2 µm for capillaries, 1 µm to 20 µm for collagen fibril bundles and approx. 100 nm for collagen fibrils [5–7]. Dependent on the cell type both positive, convex, and negative, concave, curvatures can be of interest.

Several previous studies have focused on three dimensional curvature in the nano-meter scale[8–11] using for instance electro-spun fibers. Yet structures with curved surfaces in the orders of several micrometers are less studied, whereas not less important. Cellular responses and the underlying mechanism of curvature sensation in these micrometer dimensions are not yet fully understood. Systematic experimental studies are rare but would help to reveal the molecular mechanism of curvature sensing and thus generate new insights in how cell behavior can be controlled and triggered using curved topographical features.

Based on our assessment, reasons for the lack of studies result from limitations of the commonly used microfabrication methods, like wet etching or reactive ion etching. Such methods can be costly and ate typically suitable for the generation of sharp edged surfaces and structures with low controllability over curvature[12-13]. Other molding methods, like fiber assisted molding[14] or the gel trapping technique[15] are often restricted to the generation of tightly packed surface topographies.

Addressing the lack of surfaces with defined but varying curvature features suitable for systematic in vitro studies, we generated a topography chip system. The

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spacing between the curvatures, the curvature radii as well as the dimensions and complexity of curved surfaces can be varied in a systematic manner. The versatile chip platform is produced by a combination of photolithography with thermal reflow[16] followed by PDMS molding and if needed hot embossing, to transfer the structure in different polymer materials.
The CurvChip facilitates the systematic investigation of cell responses to substrate curvature in a physiological interesting size range. Due to its easy handling as chip system it is suitable to perform systematic studiers of various cell types like epithelial and smooth muscle cells, endothelial cells, osteocytes or stem cells. Such studies will not only help to further understand the mechanism of curvature sensation but can also contribute to the optimization of biomaterials for applications in regenerative medicine.

2 Materials and Methods

Photolithography with thermal reflow. The photolithographic process used to generate the curved surface topographies is illustrated in *figure 1*.

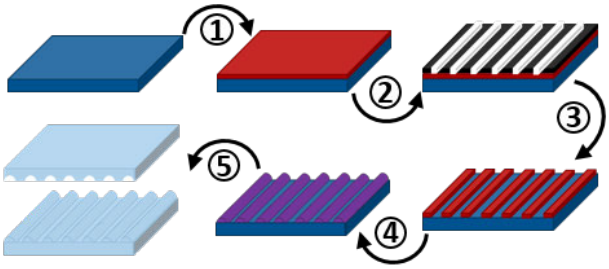

Figure 1: Depiction of soft lithography with photoresist thermal reflow for the generation of rounded surface structures.

An in-house constructed, low-cost, bench-top photolithography station with laminar flow hood is used [17]. To fully passivate the surface of cleaned wafer (Siegert Wafer GmbH) and to remove adsorbed water, the wafer were heated to 200°C for at least 30 minutes. Subsequently HDMS (hexamethyldisilazane) was spin coated onto each wafer (KLM SCC-200, Spin coater 1.4). Afterwards the positive photoresist ma-P1275 HV (microresist technology) was spin-coated according to the wanted layer thickness. The resist was dried in a soft bake step at 120°C. To generate the microstructure a photomask with the wanted structure is placed onto the wafer (contact mode). The wafer, together with the mask is then exposed to UV light at 375 nm. For the development of the structure the developer ma-D 331 (microresist technology) was used. To yield curved topographies from classical sharp edged lithographic structures, the wafer is slowly heated above the glass transition point of the photoresist (here 140°C) to allow thermal reflow. Based on the surface tension of the resist the structure melts within the restriction of the original structures. An initial aspect ratio of approx. 1:3 is (height : width) leading to the formation of semi-spheres or semi-cylindrical topographies. The so formed curved patterns solidify when cooled back to room temperature and can then be used for further molding procedures.

Repetitive molding with polydimethylsiloxane. To transfer the structure to polydimethylsiloxane (PDMS), we mixed Sylgard 184 (Dow Corning) curing agent and base in a ratio of 1:10 and casted it onto the wafer. The mixture was then cured at 80°C for not less than 1h. The hardened PDMS was detached from the wafer and then stored at 80°C overnight to fully cure the PDMS. To generate the initial convex pattern of the wafer, the PDMS was then molded again using the same mixture of Sylgard 184 and cured at 80°C for not less than 1 h. The two layer of PDMS were detached from each other, yielding both concave and convex curved structures

Hot embossing using PDMS mold. To generate a more versatile chip system with variable surface properties hot embossing can be used. We use a variation of the process described by Goral et al. [18] using a polyvinylchloride (PVC) microscope slide (Rinzl, thickness approx. 0.4 mm), a PDMS molding (approx. 0.1 mm), two glass microscope slides and ¾'' binder clips, as well as an oven heated to 100°C.

Scanning Electron Microscopy (SEM) examination. Samples were prepared on 90° sample holder and sputter-coated with gold (Polaron Equipment Limited, SEM coating unit E5100). Examination was made at 5-10 kV (Zeiss, DSM 962) either at 0° or 30° tilting.

Cell culture. Human dermal Fibroblasts were cultured on O_2 plasma treated PDMS which was incubated with 20% fetal calf serum (FCS, Gibco) for approx. 10 hours prior cell seeding. Medium (Dulbecco's modified eagle medium with 10% FCS and 1% Penicillin/Streptomycin, all purchased from Gibco) was exchanged every 2-3 days.

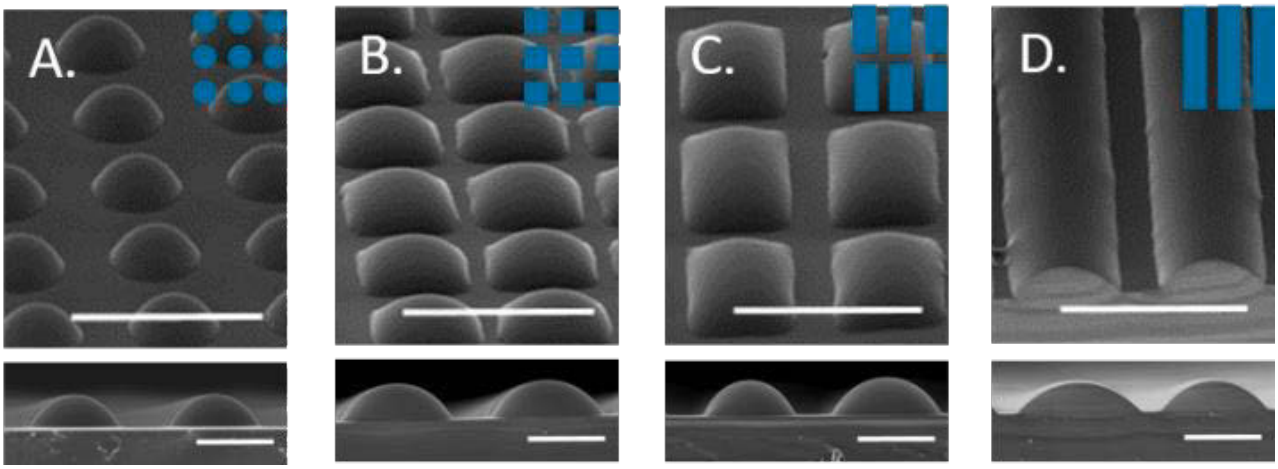

Figure 2: SEM images of tilted view (large) and breaking edge (small) of double molded PDMS structures sputter-coated with gold. Blue structures indicate photomask layout. **A.** circular basic form, **B.** square basic form, **C.** rectangular basic form, **D.** linear basic form. Scale bar, tilted view 100 µm breaking edge 40 µm.

3 Results

Using low-cost variations of commonly used techniques, including photolithography with thermal reflow, subsequent (repetitive) PDMS molding and hot embossing we are able to generate a highly defined topographical chip platform with various kinds of three dimensionally curved structures. Topographies can range from partial spheres (*figure 2A.*), over partial cylindrical rods of various length (f*igure 2B*. square basic form or f*igure 2C.* rectangular basic form) to longer and more complex structures (f*igure 2B.*) by only changing the photomask layout (illustrated in the blue structures in *figure 2*). Different patterns were generated in a reproducible way on silicon wafers up to size of 3''or for rectangular chips matching the size of standard microscopy slides.

We are able to independently vary the spacing between the curved topographies and the curvature itself, accomplishing to cover a broad range of possible applications. Additionally, using the hot embossing approach, we are capable of generating chips with various surface chemistries and material properties, exemplary presented in *figure 3* by hot embossing of PVC microscope slides with microstructures. First cell experiments show the suitability of the chip platform for investigating cell responses (see *figure 5*).

4 Conclusion and Outlook

We describe the fabrication of a highly defined topography chip system with variable convex and concave 3D curvatures to systematically investigate the general impact of substrate curvature on the cell function using low cost methods.

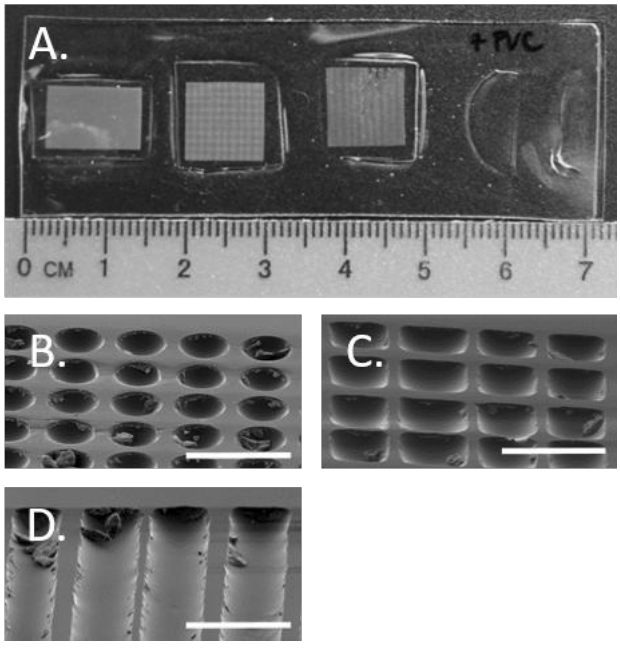

Figure 3: Hot embossed PVC. A. Image of microstructured PVC microscope slide, **B.** SEM image of microstructure with circular basic form, **B.** SEM image of microstructure with square basic form, **C.** SEM image of microstructure with linear basic form. SEM images are tilted views and sputter-coated with gold, scale bar 100 µm.

In order to separately test, for instance, the effect of topography on stem cell differentiation or the impact of pharmacologically active substances on curvature sensation, the chip platform can also be used like a multi-well chamber (see *figure 4*), enabling a high throughput screening approach.

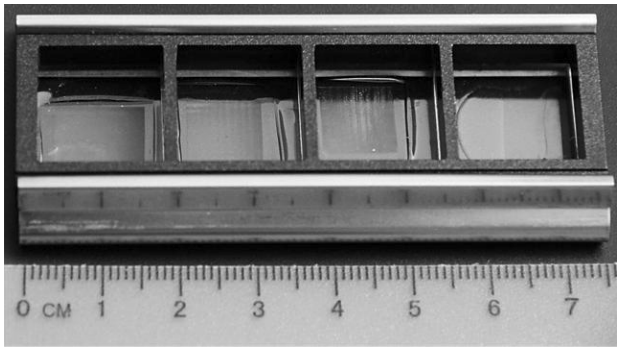

Figure 4: Chip platform with multi-well attachment. Attachment here illustrative ProPlate by gracebio.

Figure 5 gives two examples for the response of cells to rounded microstructures*: A.* Arrangement of the nucleus over spherical cavities and *B.* alignment of the cells along the axis of the convex partial cylinder.
Detailed mechanisms of cell responses are not understood but surface features are a promising tool to influence cells and tissues. The CurvChip allows to study reposes of cell to curved features in a high-throughput fashion and, therefore, providing a useful tool for basic cell and applied biomaterial research.

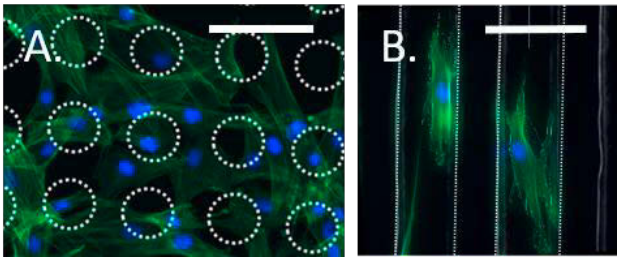

Figure 5: Examples for effects PDMS microstructures have on human dermal fibroblasts. A. Microcavities approx. 50 µm in diameter. Cells orient their nucleus over the cavities. **B.** convex partial cylinder approx. 60 µm in diameter (radius of curvature approx. 26 µm). Cells show alinotaxis. Cells are fixed and stained for actin (ActinGgreen 488, green) and nucleus (NucBlue, blue), scale bars 100 µm.

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