Renal Epithelial Cell Responses to Supramolecular Thermoplastic Elastomeric Concave and Convex Structures

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The nephron naturally provides a concave conformation for epithelial cells, yet biomedical applications such as bioartificial kidney can employ convex seeding. Frequently glass or polydimethylsiloxane are utilized as base materials to study renal epithelial cell response to curvatures. Insights on relevant materials for biomedical applications remain limited. Here it is investigated how human immortalized renal proximal tubule epithelial cells (RPTEC) respond to a range of concave and convex curvatures made from a bis-urea modified polycaprolactone material. Solvent cast chips containing a 50–500 µm diameter range of both concave and convex semicircular structures are successfully produced. Concave structures are completely covered by cells under all conditions. Yet cell layers present gaps on the summits of convex structures; the relative gap size enlarges as the diameter decreased. Increased proliferation time and cell seeding density allow for cells to overcome summit avoidance and nearly engulf convex structures. Interestingly, sample inversion also results in gap closure on convex structures during live imaging. Polarization of RPTECs is more prominent on concave compared to convex structures. These findings suggest that biomedical applications such as the bioartificial kidney should focus on concave seeding of cells to acquire more functional cell monolayers.

1. Introduction

Cells have the ability to sense and integrate geometrical, mechanical, and chemical cues from the surrounding microenvironment through a complex interplay of substrate binding, cytoskeleton remodeling, and other mechanosensitive processes.[3–11] Curved geometries are of particular interest as they reflect the in vivo cellular environments, which are rich in curves, such as in blood vessels, cornea, and nephrons. In recent years, our comprehension of cellular curvature response has made significant strides.[4–11] Two types of curved structures can be distinguished, concave and convex, each eliciting different cellular responses in differentiation,[4] adhesion,[5,12] migration,[9,13] and alignment.[6,8,13,14] Cells have the capacity to sense curvature magnitudes larger than the size of the cell.[6,14] A combination of cells nuclear deformation, cytoskeletal organization, cellular contractility, and level of cell–cell contacts likely determine the cell response.[5,8,14,15]

A growing body of literature employed renal epithelial cells (RECs) on curved substrates to understand epithelial response toward curvature.[5,8–10] RECs naturally reside in a concave environment of the kidney’s nephron. Elucidating the response of epithelial cells on curvatures helps in understanding natural processes such as tubulogenesis, but also aids in the development of tissue engineering approaches such as the bioartificial kidney.[9,16–18] The concept of the bioartificial kidney entails an array of hollow fiber membranes, which are lined with RECs to improve filtration during hemodialysis treatment.[16] Inner or outer seeding of RECs on hollow fibers is currently governed by practical constraints, smaller inner diameters prove challenging for inner seeding.[16,17] These seeding locations result in different cell configurations, i.e., concave or convex, respectively. There are indications that cell adhesion decreases on smaller convex fibers.[17] Differing results have been reported on the effect of curvature configuration on functional polarization of RECs. Yu et al. claimed that REC polarization increased on convex structures over concave structures.[8] However, Shen et al. showed polarized expressed transporters and enzymes to increase their activity with increasing concave curvature.[18] However, there is a clear scale difference between both studies, Yu et al. employed...
a physiological curvature range, while Shen et al. employed a larger curvature range found in biomedical applications.\(^{[8,18]}\) These differing scales and results validate further systematic investigation of REC response to convex and concave structures.

As pertinent, is the acquisition of insights into the cellular behavior of RECs on relevant curved biomaterials. To date cell curvature response has been mainly studied on glass or polydimethylsiloxane (PDMS), due to their advantage processibility, availability, and cost. However they are not suitable for the design of a bioartificial kidney due to difficulties in the tunability of degradability, the introduction of bioactivity and the challenges in hollow fiber processability.\(^{[5,8–10]}\) The response to thermoplastic elastomers is more relevant, as mainly thermoplastic membranes are employed in bioartificial kidney research, and present desired properties.\(^{[19]}\)

Therefore, we set out to investigate the response of RECs to a range of concave and convex structures in a supramolecular material based on polycaprolactone modified with supramolecular bis-urea moieties (PCL-BU). PCL-BU was selected as a base material due to its biocompatible nature and its processability into membranes (Figure 1A,B).\(^{[20–22]}\) Moreover, the bis-urea motifs permit modular introduction of bioactive components in the base material to steer cell behavior.\(^{[22,23]}\)

Research performed on REC sensing of curvatures has been primarily executed with either Madin Darby Canine Kidney (MDCK) and/or Human kidney 2 (HK-2) cells.\(^{[5,8–10,18]}\) However, HK-2 cells have lost the expression of several transporters compared to other human REC cell lines, which are crucial to build a bioartificial kidney.\(^{[24–28]}\) The hTERT immortalized renal proximal tubule epithelial cells (RPTECs), with superior transporter expression, were selected to systematically study RECs response to curvature on PCL-BU.\(^{[25–27]}\) PCL-BU based chips were produced encompassing semicylindrical structures with diameters within the physiological range (50–70 \(\mu m\)) up to those found in biomedical applications (250–500 \(\mu m\); Figure 1B).\(^{[17,18,29]}\) Flat areas were employed as controls. Chips were coated with

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Overview of base material, chip design, and micrographs. A) Chemical structure of bis-urea modified polycaprolactone, the employed base material of the chip. B) Schematic representation of the employed curvature mold. Semicylinders were employed with a diameter ranging between 50 and 500 \(\mu m\). The height or depth of a structure was set to the radius with a maximum of 175 \(\mu m\), ergo the width of the 500 \(\mu m\) diameter semicylinder is set to 477 \(\mu m\). C) Scanning electron micrographs of chip, concave structures (top row), and convex (bottom row) depicted. Scale bars are 100 \(\mu m\).
L-3,4-dihydroxyphenylalanine (L-DOPA) and collagen type IV to ensure RPTEC attachment.[15] The cell response to the curvatures was determined from cell coverage, cell polarization, and collective cell migration response. As REC naturally reside in a concave environment, the formation of a confluent polarized RPTEC monolayer in the concave structures is expected. Convex structures are hypothesized to induce avoidance by cells, which might be overcome by increasing cell–cell contacts.

2. Results

2.1. Successful Production of PLC-BU Based Curvature Chip

Previously in-house developed curvature chip technology enabled successful fabrication of PCL-BU chips through solvent casting in PDMS molds (Figure 1C).[14] Secondary striation structures were visible on all concave and convex structures (Figure 1C). An second mold produced from a chip that contained an additional thin PDMS coating produced PCL-BU chips without secondary structures, but only with a viable size range from 250 μm and higher (Figure S1, Supporting Information).[14] Thereby the chip without secondary structures did not enable the study of the desired broad range of curvatures. The semicircular geometries on the chip had a designed maximum height of 175 μm; larger cylinders were lowered in the mold design to match this height to enable confocal microscopy. In general, the diameter of the cylinders on the PCL-BU chip was reduced compared to the mold diameter (Table 1). Small discrepancies were observed between concave and convex structures, with the exception of concave 50 μm which showed a larger discrepancy of 14 ± 2 μm. Hence forth, the mold diameter will be used as nomenclature in the study to refer to the cylindrical diameter range.

2.2. Preferential Cell Coverage and Polarization on Concave over Convex Curvatures

Cells were cultured on the chips presenting the broad curvature range from 50 to 500 μm. RPTECs were seeded at an initial density of 4 × 10⁴ cells per chip, which corresponds to 80–90% confluency on flat substrates. Flat control surfaces and all concave structures showed full coverage by RPTECs after 5 days of culture (Figure 2A). Extending the culture period to 10 days did not alter cell coverage on flat surfaces nor on concave structures (Figure 2B); overall a slight incremental trend in cell number was observed (Figure 2C). Cell layers cultured on convex structures presented gaps at the tops at 5 days. Structures with higher convex curvatures (i.e., smaller diameter) showed a larger relative gap size compared to lower convex curvatures at 5 days of culture (Figure 2A,B). Gap size is defined as the percentage of space unoccupied by cells on the curved structures. These gaps were shown to reduce in size when the culture period was prolonged to 10 days (Figure 2A,B). A slight increase in cell numbers accompanied the decrease gap size on these convex substrates (Figure 2C). RPTECs were cultured on the smoothed chips with a curvature range between 250 and 500 μm to exclude potential interference of contact guidance by the secondary striation structures on cell response. RPTECs showed similar cell coverage on smoothed chips compared to the rougher chips at 5 days of culture (Figure S2, Supporting Information).

Primary cilia formation was assessed to determine the level of apical polarization of RPTEC on all structures, through alpha-tubulin staining. Overall, flat and all curved structures showed an incremental trend in primary cilia positive cells between 5 and 10 days culture (Figure 2A,D), with the exception of convex 50 and 70 μm, and concave 50 μm structures. Primary cilia formation was more pronounced on concave structures than on convex structures with diameters of ≥ 70 μm at 10 days of culture (Figure 2A,D). Interestingly, alpha-tubulin expression was low on the summit of convex structures after 10 days of culture (Figure 2A); this behavior was also sporadically observed in cells cultured on flat substrates (Figure S3, Supporting Information). Cilia can still be observed in cells which did not strongly stain positive for alpha-tubulin (Figure 2A; and Figure S3, Supporting Information).

2.3. Cell Density Affects Summit Avoidance

A confluent cell monolayer on hollow fibers is key for the bioartificial kidney to act as an selective membrane.[16] An increase in overall cell number likely explains the observed increased coverage of convex structures at 10 days compared to day 5. It has been proposed that increased cell–cell contacts (i.e., increased cell density) impose cells to cover unfavorable convex structures due to a lower level of cellular freedom.[5] To further explore this notion, cells were seeded at quarter or double densities on convex structures compared to previous experiments. We hypothesized that convex structures with higher curvatures are avoided by RPTECs at lower seeding densities and overgrown with increasing cell numbers. At a quarter cell density, no single cells were observed on the summit of convex structures with a diameter of ≤100 μm (Figure 3A). At convex diameters of ≥250 μm single cells could be observed on the summit (Figure 3A). Both single cells and colonies could be observed in all concave structures, with cells accumulating in the center of the larger structures (Figure S4, Supporting Information). Increasing the cell density showed an increase in cell coverage on all convex structures compared to the initial cell density, thereby decreasing the gap size compared to initial seeding density (Figures 2 and 3A,B). Increased cell density did not alter the cell coverage toward concave structures compared to the initial cell seeding density (Figure 2A; and Figure S4, Supporting Information).

Table 1. Actual curvatures diameter. The diameters of the designed mold versus the diameters measured by scanning electron microscopy on the polycaprolactone based chip. Mean ± standard deviation in μm, n = 3.

| Mold diameter [μm] | 500 (a) | 350 | 250 | 150 | 100 | 70 | 50 |
|---------------------|--------|-----|-----|-----|-----|----|----|
| Concave diameter [μm] | 464 ± 3 (a) | 305 ± 5 | 214 ± 4 | 141 ± 2 | 92 ± 3 | 56 ± 3 | 36 ± 2 |
| Convex diameter [μm] | 499 ± 4 (a) | 328 ± 11 | 233 ± 9 | 132 ± 11 | 93 ± 8 | 67 ± 5 | 52 ± 3 |

(a) Corrected for the lowering of the cylinder in the mold design to uphold height maximum of 175 μm. Actual mold width was 477 μm at this height, the measured width was 430 ± 3 and 467 ± 4 μm for concave and convex, respectively.
2.4. Chip Inversion Influences Curvature Response

Collective RPTEC migratory behavior was investigated to assess whether there are fluctuation in monolayer confluency on curved structures in real time. Cells were monitored from day 4 until day 5. Live imaging required sample inversion. To decouple the influence of live imaging and inversion, a control experiment was performed where the sample was inverted within the original well after 4 days of culture and left for 1 day in the new position. Unexpected behavior was observed as the gaps on the summits of convex structures closed during live imaging (Figure 4A). Concave structures remained lined with
cells over time. An incremental trend in gap closure velocity was observed as the convex diameter increased, with a significant increase between 50 and 500 µm (Figure 4B; \( p < 0.05 \)). A single sample of 150 and 500 µm presented small defects in the monolayer away from the suture over time resulting in a reportable gap size (Figure 4C). Gap size was shown to decrease on structures with \( \leq 250 \) µm diameters after inversion in the control experiments (Figure 4C). However, complete gap closure on all convex structures was not mimicked in the inverted control experiments compared to experiments after live imaging.

3. Discussion

In this study, we set out to investigate the response of human RPTECs toward a range of concave and convex structures on a relevant biomaterial. The previously described curvature chip by Werner et al. was based on mold technology; this enabled us to alter the base material from commonly studied PDMS to biomaterials more relevant for biomedical applications. Solvent cast curvature chips were successfully produced from PCL-BU, which is a biocompatible supramolecular thermoplastic elastomer. The reduction observed in the structures’ diameter on the chips compared to the mold, most likely originates from shrinkage stress, which is commonly observed when polymers are solvent cast. Despite the reduction in size, the overall range in size differences was conserved. Previous studies have shown that different renal epithelial cell lines align perpendicular to the cylinder axis on convex structures and parallel to the cylinder axis on concave structures, both below 100 µm. Secondary features observed on the curvature chip are most likely capable of steering cellular alignment by contact guidance, thereby...
confounding orientation analysis. However, convex avoidance was not altered by these secondary features.

RPTECs lined all concave and flat surfaces, yet convex surfaces showed gaps in the monolayers at the summits. The relative area clear of cells increased with increasing curvature (i.e., decreasing diameter). The summit was left unpopulated by RPTECs on convex structures with a diameter \( \leq 100 \, \mu m \) with the initial cell seeding density at 5 days. A RPTEC has an estimated size of around 20 \( \mu m \), thereby indicating that cells sense curved structures many magnitudes larger than themselves, in line with previous research findings.[6,14]

In similar work to our study, performed by Yu et al., convex structures presented summits overgrown with cells. However, these experiments were performed after full confluence was reached, with a larger different REC type.[8] Moreover, Pieuchot et al. noted that larger epithelial colonies showed decreased convex avoidance, and we observed a slight increase in the number of cells on convex structures as the gap size was reduced between day 5 and 10.[5] This led us to hypothesize that convex avoidance by epithelial cells could be altered through changes in cell density. Potentially epithelial layers might redistribute forces compressing the nucleus through their actin cytoskeletons, which are linked by cell–cell contacts, or overcrowding might force cells on to the convex structures which are preferentially avoided. In our results, colonies and single cells could be observed on convex structures \( \geq 250 \, \mu m \) at lower cell densities. Indicating smaller convex structures to be avoided. Complementary, an increase in cell density reduced the summit avoidance of the convex structures by RPTEC. In terms of achieving a confluent cell monolayer in the bioartificial kidney design, the results imply that outward seeding will require more cells to overcome geometrical constraints than when seeding inside the hollow fiber.

Polarization was determined by the formation of primary cilia, which are crucial for sensing apical flow by RECs.[32] Flow in turn has been shown to be crucial for polarized transporter expression.[33] In our results, primary cilia formation was more prominent on concave structures compared to convex. It remained inconclusive if polarization increased as curvature decreased. The decrease in alpha-tubulin expression observed on convex, and sporadically on flat substrates, remains puzzling and might be due to postmodification or truncation of binding epitope of the antibody. Previous studies on the effect of curvature on REC polarization yielded similar and differing results. Functional data of Shen et al. indicated that polarized expressed transporters and enzymes increased in activity as the concave diameter was decreased from 1200 to 400 \( \mu m \).[18] On the other hand, Yu et al. showed improved polarization of

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**Figure 4.** Migration on curvatures. A) Stills from live imaging at different time points. RPTECs were cultured for 4 days on curvature chip, stained with CellTracker Green and imaged for 12 h with the chip inverted from the original culture position to enable imaging. Scale bars are 50 \( \mu m \), curvature diameter of 150 \( \mu m \) selected as representative situation. B) Gap closure velocity as a function of convex diameter. The gap closure velocity was determined from the linear section of the gap closure graphs as a function of time. Mean \( \pm \) standard error of the mean, \( n = 3 \). Inlay graph is an example and depicts representative gap closure curve on 150 \( \mu m \) convex structure in which the linear gap closure speed is highlighted. This speed is plotted in the larger graph. C) Control experiment on the effect of imaging on curvature response. Graph depicts: cells cultured for 5 days on curvature chip (white; Control; data replotted from Figure 2 for convenience), cells cultured for 4 days after which chips were inverted and cultured for 1 day (black; Inverted control), and gap size at the end of live imaging experiment (gray; Live imaging). Gap size was determined by measuring the cell coverage by f-actin staining. Mean \( \pm \) standard error of the mean, \( *p < 0.05 \) compared to 50 \( \mu m \), \( n = 3–4 \).
apical Zona Occludens 1 and basal NaK-ATPase α1 on semicylindrical convex structures than on concave structures (both a diameter of around 90 µm) and flat surfaces.[36] This leads to the unanswered question why REC polarization is equal or better on convex over concave structures within a physiological diameter range, taking into account that REC naturally reside in a concave environment.[29] Several parameters might be causal to the discrepancy between the work of Yu et al. and ours. First, different REC lines were employed, MDCK and HK-2 versus RPTEC. Difference in intrinsic cellular contractility might play a role, a factor which has been shown to affect cellular response to curved structures, potentially this includes polarization.[8,34] Second, different materials were employed, i.e., PDMS versus PCL-BU. These materials differ in surface chemistry and stiffness (3 vs 30 MPa)[8,35] Both have been shown to alter the cell maturation.[36,37] It would be an interesting topic for future research to investigate if material properties can switch preferred polarization environment between convex and concave. Finally, Yu et al., Shen et al., and our study all employed different polarization markers, follow up research should investigate which polarization markers are most resilient in describing the polarized nature of a REC.

Taken together, the results of Shen et al. and ours indicate that biomedical applications employing hollow fiber membranes such as the bioartificial kidney potentially benefit from concave seeding of REC to improve cell polarization; though a more in-depth study is required on the effect of curvature on REC polarization. For a final bioartificial kidney design has to be considered whether improved polarization and reduction in required cell numbers outweighs the difficulties faced with inner cell seeding.

An unexpected phenomenon was observed during live imaging, when the gaps in cell layers on convex structures closed. This behavior could be reproduced with sample inversion and static evaluation of the samples without subjecting them to the live imaging procedure, albeit to a lesser extent. Gap closure speed reduced with increasing curvature. Competing convex avoidance effect might have reduced the gap closure speed. That being said, others have not reported strong changes in cell migration speeds on structures within the 50–500 µm diameter range.[4,10,14] Alternatively, it might be that increased amount of cells on 500 µm increase the collective migration speed. Sales et al. found indications that sample inversion reduced contact guidance. They speculated that inversion induces a small pulling force on internal structures, which allows for more random actin polymerization not dictated by substrate structures.[38] This would suggest that gravity might be able to alter curvotaxis, yet Pieuchot et al. showed that sample inversion did not affect single cell convex avoidance.[5] Moreover, Jansen et al. performed outward seeding of RECs on hollow fibers and did not report polarized accumulation of cells.[7,9] This leads us to conclude that the observed gap closure is likely influenced by live imaging on inverted structures. Future investigation should explore this observation further to assess what are the underlying mechanisms and if it has consequences for cell migration data acquisition on curved substrates.

The bis-urea motifs in the PCL-BU allow for the modular integration of bioactives in the chip during casting.[22] Biofunctionalization of curvatures opens up the possibility to manipulate curvature sensing by cells. Functionalities such as cell–cell contact mimicking peptides might be potent in eliciting a different cell response toward curvatures, as we observed cell density to be crucial for epithelial response to convex structures. Alternatively, cell adhesive Arg-Gly-Asp peptides could be applied, as they affect for example cell migration.[21,40] This can be complemented with more in-depth investigation of the cell curvature response by evaluating curvature related cellular processes, such as cytoskeletal dynamics, lamina expression, Bin/amphiphysin/Rvs modulation, or yes-associated protein 1/transcriptional coactivator with PDZ-binding motif localization.[5,14,15]

4. Conclusion

In this study, the response of human RPTECs toward a range of concave and convex structures based on PCL-BU was assessed. Employing PCL-BU enabled the study of curvature response to a relevant biomedical material compared to the often utilized PDMS. PCL-BU based chips were successfully produced with semicylindrical structures ranging from 50 to 500 µm in diameter (RPTECs are around 20 µm in size). RPTECs completely lined concave structures, and showed improved polarization compared to convex structures. The summits of convex structures were increasingly avoided by cells as the curvature increased. Increased culture time and higher cell numbers allowed RPTEC to overcome summit avoidance and thereby near complete colonization of convex structures. The insights obtained in this study can be applied for in vitro models or biomedical applications such as a bioartificial kidney, which should focus on concave seeding of cells to acquire more confluent polarized cell monolayers.

5. Experimental Section

Chip Production and Characterization: Chip molds contained semicylindrical convex and concave structures with diameters of 50, 70, 100, 150, 250, and 500 µm and respective principal curvatures of $\kappa = 1/25, 1/35, 1/50, 1/75, 1/125,$ and $1/250$ µm$^{-1}$. Rectilinear pillars were positioned at the bottom of the well as the mediating structures. All chips were produced as described by Werner et al.[14] PCL modified with bis-urea motifs (PCL-BU, $M_n = 2.7$ kg mol$^{-1}$ per segment, SyMO-Chem) was dissolved in hexafluoroisopropanol (Fluorochem) for 1 day to produce a 100 mg mL$^{-1}$ casting solution. 3 mL of casting solution were gently poured on the mold and left to evaporate for over 2 days at RT. Chips were characterized by scanning electron microscopy (SEM; Quanta 600, FEI) under high-vacuum with an applied voltage of 1–2 kV and a spot size of 3.0 nm.

Cell Culture: PCL-BU based chips were UV-sterilized for 10 min and subsequently incubated in phosphate-buffered saline (PBS) for 10 min. 2 mg mL$^{-1}$ L-DOPA (Sigma-Aldrich) was dissolved in 10 × 10$^{-3}$ M Tris-buffer (pH 8.5, Merck) for 1 h at 37 °C. The solution was sterile filtered and applied on the chip for 4 min at 37 °C. Cells were washed with PBS and a secondary coating of 25 µg mL$^{-1}$ Collagen type IV in PBS (derived from human placenta, Sigma-Aldrich) was incubated on the chips for 30 min at 37 °C. Secondary coating solution was aspirated and washed with PBS. The rectangular chips were placed in a custom made PDMS well with a culture area of 3 cm$^2$. RPTEC cells (RPTECs-TERT1; ATCC) were cultured in complete medium consisting of DMEM:F-12 Nutrient Mixture (Gibco), L-Glutamine and 15 × 10$^{-3}$ M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Furthermore, the medium was supplemented with 1 v/v% penicillin-streptomycin.
(Invitrogen), RPTEC growth kit of ATCC (PCS-999-058, PCS-999-059) and 0.1 mg mL⁻¹ C418 (Sigma-Aldrich) in a humidified atmosphere at 37 °C and 5% CO₂. Cell passages between 3 and 20 were employed in this study. RPTECs were seeded either 0.33 × 10⁶ cells cm⁻² (quadrant density), 1.33 × 10⁶ cells cm⁻² (normal density), or 2.66 × 10⁶ cells cm⁻² (double density) and cultured for either 5 or 10 days, with medium changes every 2–3 days.

**Immunofluorescence Staining**: Cultures on the chip were washed with PBS (Sigma-Aldrich) and fixed with 3.7% v/v formaldehdye solution (Merck, USA) in PBS for 10–15 min. Cells were permeabilized with 0.5% v/v Triton X-100 (Merck) in PBS for 10 min after washing with PBS. Samples were subsequently washed with PBS and blocked with 5% w/v BSA (Roche) in PBS for 20 min at RT. Primary cilia were stained with anti-acetytelulin antibody (1:300, ab18251, Abcam) for 60 min in staining buffer (2% w/v BSA, 0.05% Triton X-100 in PBS) at RT to investigate cell polarization. After washing with 0.05% Triton X-100 in PBS samples were incubated with secondary antibody goat-antirabbit IgG Alexa Fluor 488 (1:200, A11008, Molecular Probes) and phalloidin atto647N (1:300, A10035, Thermo Fisher) for 45 min at RT. During the final 10 min 4',6-diamidino-2-phenylindole staining (1:500 dilution, D9542, Sigma-Aldrich) was added. Samples were washed three times with PBS. Z-stack images were acquired of each structure by confocal microscopy (TCS SP5X, Leica). Images of convex structures were processed with a custom Matlab script (v2015A) previously developed Bade et al. kindly provided by the authors.[8] This script enables to take in account the transition of a 3D object to a flat image. Gap size was defined as the percentage of space unoccupied by cells compared the full size of the structures. Gap size was quantified with TScratch (v1, ETH Zurich) in the resulting images. The phalloidin atto467N staining for fasicin was employed to identify cell boundaries, space void of cells was manually defined in TScratch. Representative images were produced by ZProjections of the z-stacks employing standard deviation projection type in ImageJ (v1.48, NIH), structures were cropped from the surrounding area. ImageJ was further used to quantify cell numbers, while primary cilia were counted blindly by two experimentalists. All experiments were performed in triplicate.

**Live Cell Imaging**: Cells were cultured under standard culture conditions until day four, after which cells were stained with CellTracker Green (1:100, Invitrogen) in culture medium for 30 min at 37 °C. Cells were washed with PBS and new culture medium was added. A custom glass bottom well was produced for live imaging. Chips were flipped and placed in the custom well kept gently in place by stainless steel clip and imaged over a 15 h time period by confocal microscopy at 37 °C and 5% CO₂. Z-stacks were acquired sequentially of each structure, an imaging cycle of all structures was completed in 30 ± 3 min. As a control experiment chips were inverted in culture wells at day four, and fixated at day five. Gap size was analyzed as stated before. All the experiments were performed in triplicate.

**Statistics**: Quantified data were subjected to a Kruskal–Wallis test with a Dunns post-test in which selections of columns were compared. Tests were performed with the use of Prism 5 (GraphPad Software Inc.). Probabilities of p < 0.05 were considered as significantly different.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

**Acknowledgements**

This work was financially supported by the European Research Council (No. FP7/2007-2013) ERC Grant Agreement No. 308045, and the Ministry of Education, Culture and Science (Gravity programs 024.001.035 and 024.003.013). Maike Werner is gratefully acknowledged for the development the curvature chip mold technology and providing it, and her input during article preparation. The authors thank Nathan D. Bade and Kathleen J. Stebe for kindly providing a custom Matlab script to process confocal curvature images.

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

biomaterials, curvatures, epithelial cells, polarization, supramolecular materials

Received: August 23, 2020
Revised: November 11, 2020
Published online: November 27, 2020

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