Supporting Information

3D Biomimetic Chips for Cancer Cell Migration in Nanometer-Sized Spaces Using "Ship-in-a-Bottle" Femtosecond Laser Processing

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Biochip design and concept

In order to develop an appropriate protocol for *in vitro* testing of the migration potential of PC3 cells in confined spaces, we calculated the total volume of the glass microfluidic platform based on the designed geometry and obtained V_T = 644 nl. All micro-reservoirs are identical: 200 µm in height and 1 mm in diameter. Hence, the volume of one micro-reservoir is: , where *r* is the radius and *h* is the height. The volumes of the channels are calculated using the formula L_n, where L_n is the length of each channel, W the width, and H the height. W and H parameters are similar for the GCh1 (~950 µm long) and GCh3 (~500 µm long) channels: ~215 µm in width and ~50 µm in height. GCh2 is ~100 µm long, ~90 µm wide and ~50 µm high. These values give , and . As a simplified representation, we have schematically drawn the biochip with the calculated volumes of each micro-reservoir and channel (Figure S1a). After loading the PC3 cells, the cells were cultured in µRs 1 and 2 until they completely occupied channel GCh1. When the cells approached the sub-micrometer channels in the panpipe scaffold (schematically shown in Figure S1b), the chemoattractant was introduced from µR4.

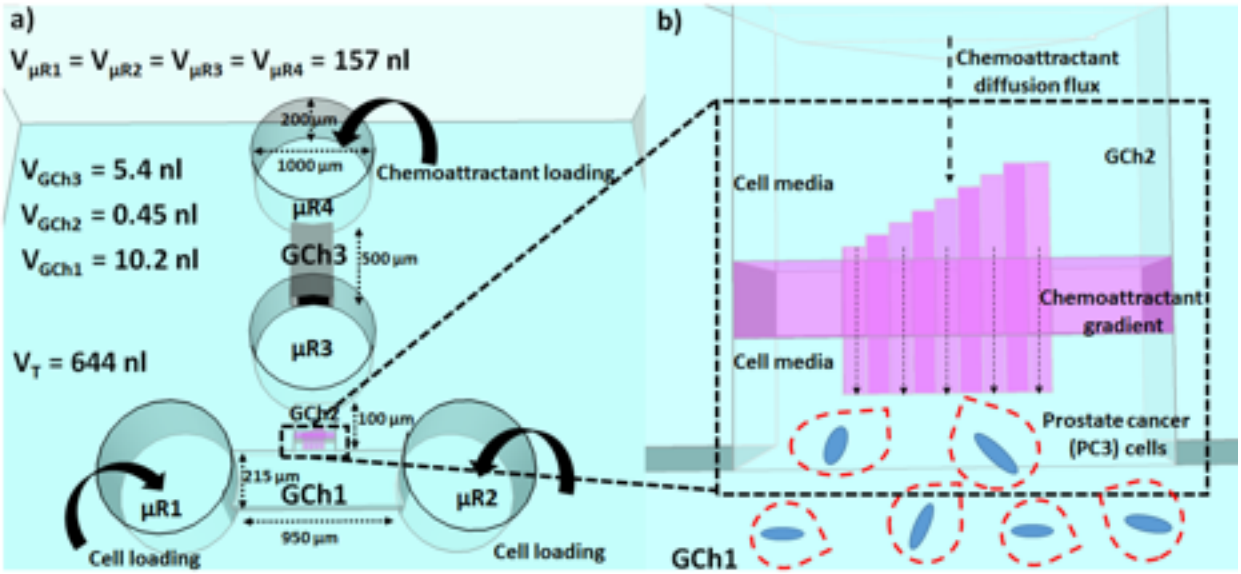

Figure S1: *Biochip volume evaluation: a) All micro-reservoirs are identical, with* $V_{\mu R1} = V_{\mu R2} = V_{\mu R3} = V_{\mu R4} = 157$ *nl. The total volume is then given by* $4 \times V_{\mu R1} + V_{GCh1} + V_{GCh2} + V_{GCh3} = 644$ *nl; b) Detailed view of the observation area and scheme for in vitro chemoattractant gradient generation for experimental testing of PC3 cell migration potential in confined spaces.*

Roughness evaluation

Roughness and surface planarity are dependent on the laser irradiation conditions and etching time. Our analyses were conducted in open channels in order to evaluate surface characteristics. The open channels, with a depth of 10 μm, were fabricated by laser scanning on a glass surface followed by thermal treatment, chemical wet etching, and a second thermal treatment.

Optimization of the laser scanning scheme is key to improving the planarity of the surface throughout the channel length. We found that line-by-line, transversal scanning with a 5 μm pitch provides the best balance for both homogenous etching of the irradiated zones and reasonable processing times at a scanning speed of 1 mm/s. Additionally, a smaller pitch of 3 μm was adopted for laser scanning at the central zones of the channels in order to avoid formation of tapered structures, since etching was always started from both ends of the channel and moved towards the center. Indeed, increasing the photon dose associated with the laser scanning pitch increased the density of crystalline zones, resulting in an increase in the etching rate.

Roughness was evaluated using the same open channels as functions of both etching time and scanning pitch. For the evaluation, six different areas on the etched surface were linearly scanned for a length of 90 μm along the channel width direction using a profilometer (see Figure S3a). After etching, we found that the surface treated at a laser power of 3 mW and a scanning speed

of 1 mm/s was quite rough, with root mean square (RMS) values of 368, 355, and 439 nm for etching times of 25, 30, and 35 minutes, respectively (data not shown). In addition to RMS, Mean Roughness (Ra) and Mean Roughness Depth (Rz) were also evaluated. Figure S2a shows the evaluation of the surface of channels after the second thermal treatment. The values decreased by one order of magnitude, and RMS values of 47, 70, and 37 nm were obtained for 25, 30, and 35 minute etching times, respectively. Thus, we conclude that the etching time has little effect on surface roughness. This result is advantageous for the fabrication of 3D microfluidic structures, since the etching time for 3D microfluidic channel formation is determined by the length and the geometry: the longer the channel and the more complex the geometry, the longer the etching time. We performed further experiments in which we modified the scanning pitch while keeping the etching time at 35 min. Figure S2b shows the roughness parameters evaluated after the second thermal treatment. The RMS values decreased from 86 to 59 to 26 nm as the scanning pitch was changed from 10 to 5 to 1 μm, respectively. We can conclude that the pitch has a more important effect on surface roughness than does etching time. Using these conditions optimized for both planarity and surface roughness, we fabricated our structures. Representative optical images of the same microfluidic structure as used in the cancer cell migration study but formed on a glass surface 35 minutes after etching and the subsequent second thermal treatment are presented in Figs. S2c and d, respectively, and clearly show that the second thermal treatment significantly decreased roughness.

Figure S3 presents image reconstruction by profilometric analysis at the intersection areas (scaffold location (a) and channel intersection area (b)) in Fig. S2d. We thus conclude that roughness in the nanometer-scale range achieved over a large-scale flat surface is applicable to the fabrica-

tion of polymeric high aspect ratio structures. Since the environment of the embedded channel was closed, an optimized etching time of 50 minutes was used, which should give the same quality of surface as in the open channels.

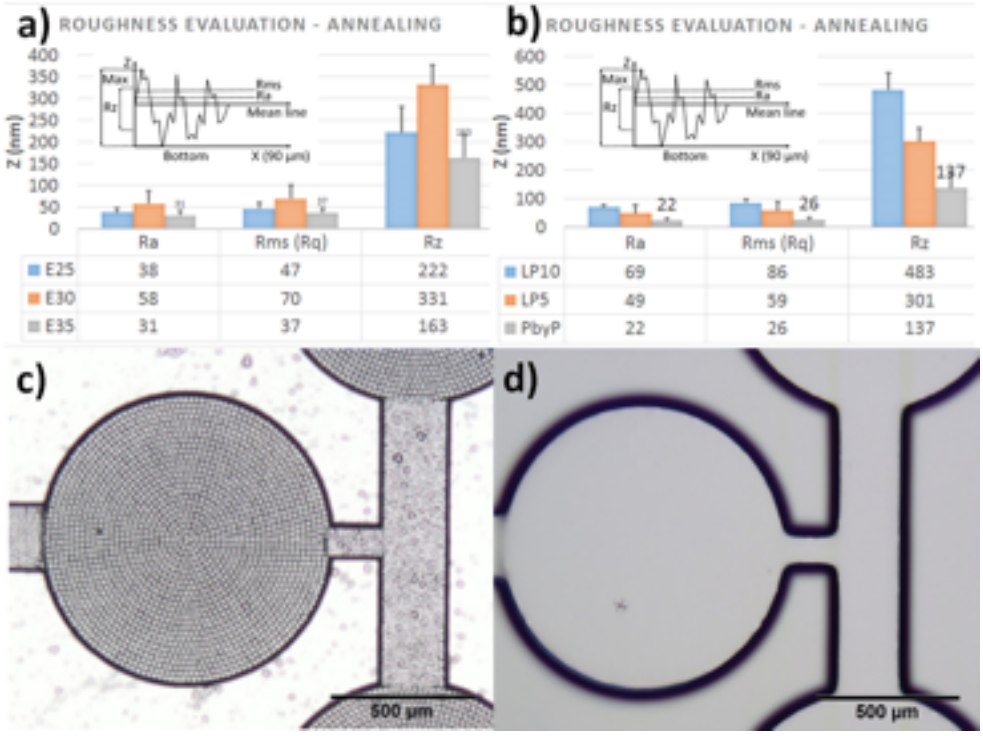

Figure S2: *(a) Dependence of the roughness Ra, RMS, and Rz parameters on etching time; (b) Dependence of the roughness Ra, RMS, and Rz parameters on scanning pitch; (c) Typical optical image of an open channel obtained 35 minutes after etching; (d) Typical optical image of an*

open channel obtained 35 minutes after etching followed by the second thermal treatment.

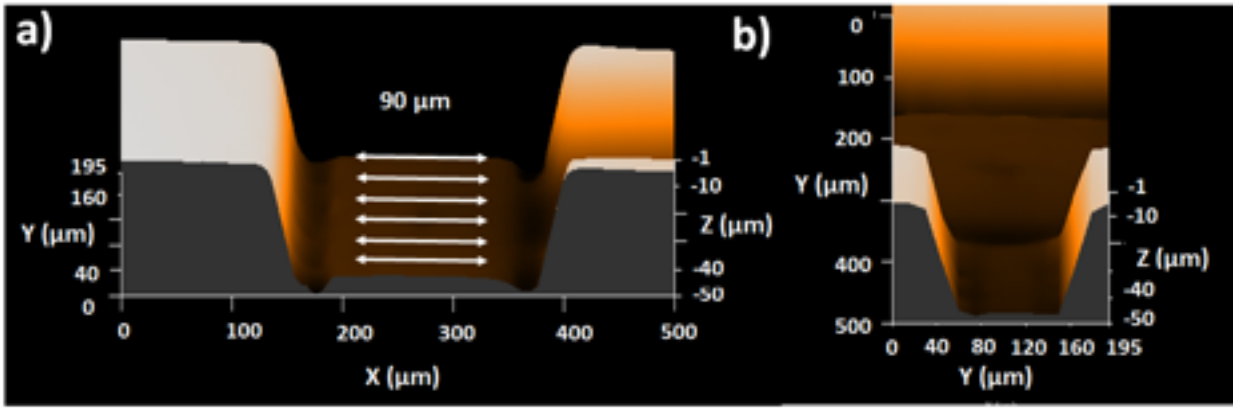

Figure S3: *Profilometry reconstruction images after the second thermal treatment (a) Scanning lines for roughness evaluation of channel surface (b) Scaffold location. Intersection of the channels.*

Experimental

A scheme showing the set-up for both FLAE and TPP is given in Figure S4.

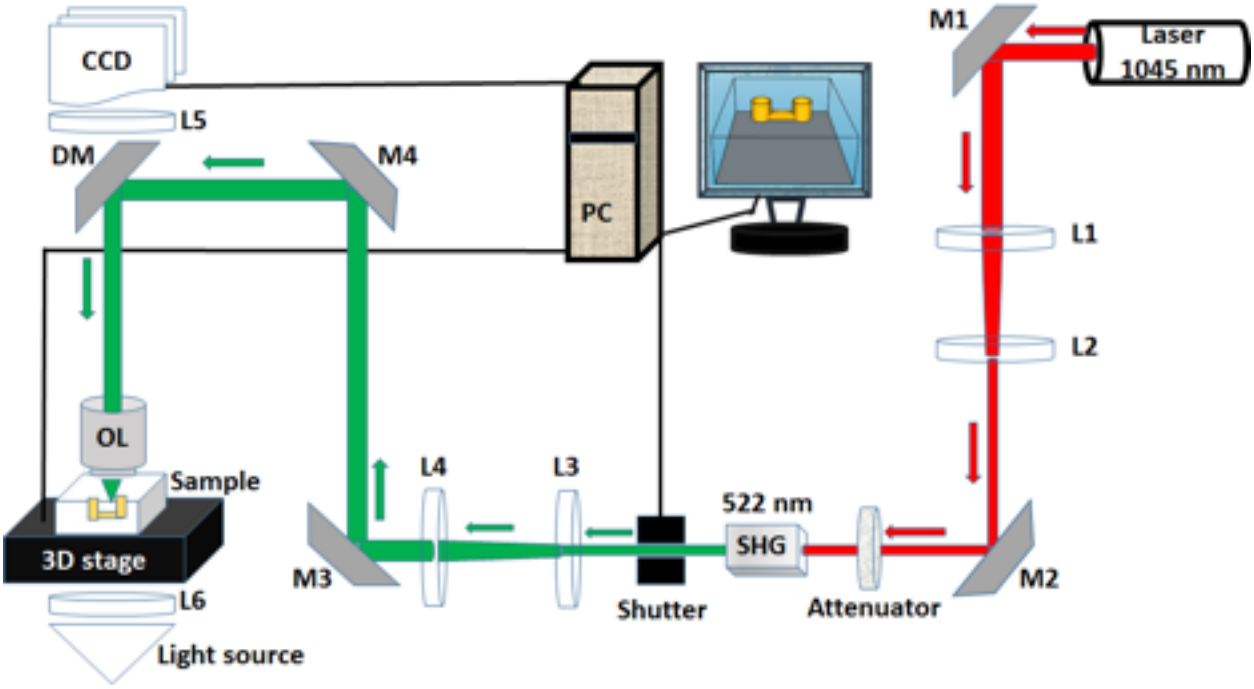

Figure S4: *Scheme showing the laser processing set-up for the "ship-in-a-bottle" FLAE – TPP process. Two lenses, L1 (f = 200 cm) and L2 (f = 50 cm), were used to reduce the diameter of the fundamental IR beam for efficient SHG generation. The power was adjusted using an attenuator. Other two lenses, L3 (f = 30 cm) and L4 (f = 300 cm), were used to expand the diameter of the generated green laser beam to match the NA of the appropriate objective lens (OL). One dielectric mirror (DM) of ultrahigh reflectivity at 522 nm allowed white light to pass through for in situ process monitoring. (M1-M4) - Silver-coated mirrors were used for guiding the laser beam. The sample was placed on a 3D stage with two stepping motor controllers which allowed light to also pass through from beneath. A 3D stage controller, shutter, and CCD camera were controlled by PC programs.*

A scheme showing the hybrid FLE-TPP process is given in Figure S5.

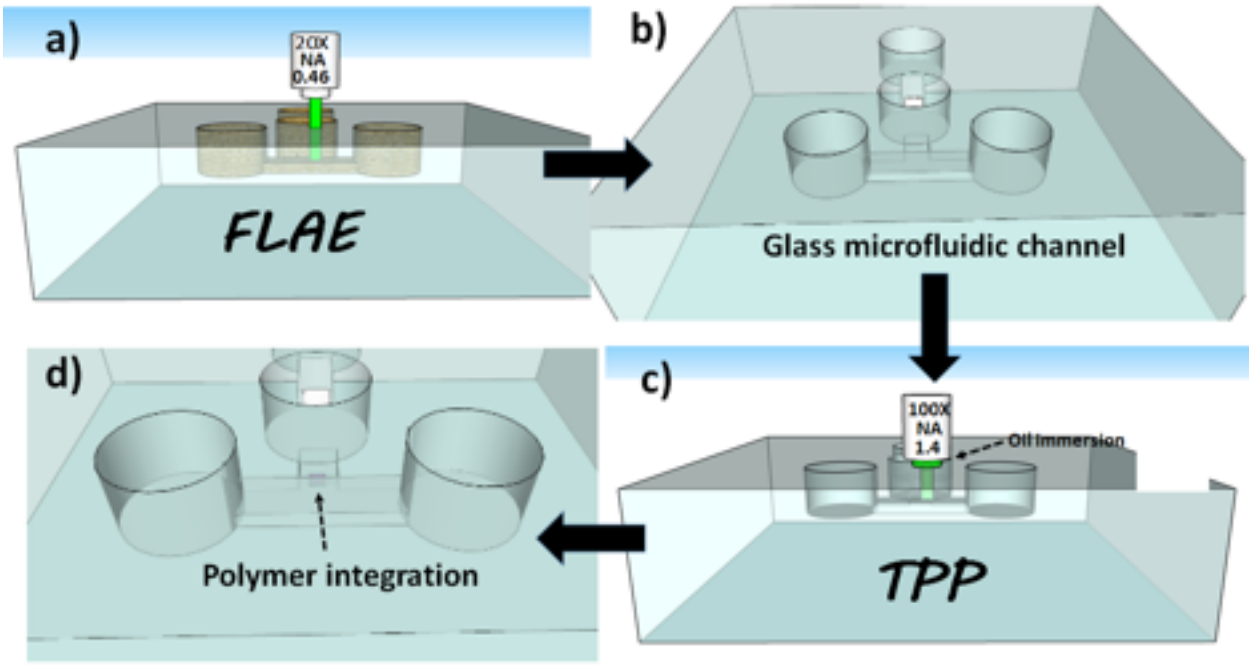

Figure S5: *Schematics showing the hybrid FLAE-TPP procedure for "ship-in-a-bottle" polymer integration (a) femtosecond laser irradiation of Foturan; (b) embedded glass microchannel developed after etching; (c) TPP of SU-8 photoresist inside a glass microchannel; (d) 3D polymeric patterns (shown by a black dashed arrow) integrated inside a microchannel.*

A ready to use hybrid biochip can be fabricated in two days: i) FLAE of glass takes about 16 hours (3 hours laser irradiation followed by 6 hours thermal treatment, 1 hour chemical etching and another 6 hours for the second thermal treatment) while ii) TPP of polymer takes about 25 hours (20-24 for prebaking and 1-2 hours processing and developing). Nevertheless, the batch process is available except for the laser direct writing process. Thus, we simultaneously treated 4 samples for the batch processes. In addition, the biochips are reusable for several experiments while the glass platform can be reused for years.

The microscope, equipped with a mini-incubator (INU-WDSM-O-F1, Tokai Hit, Japan), a CO_2 supply and temperature control at 37 ºC, is shown in Figure S6.

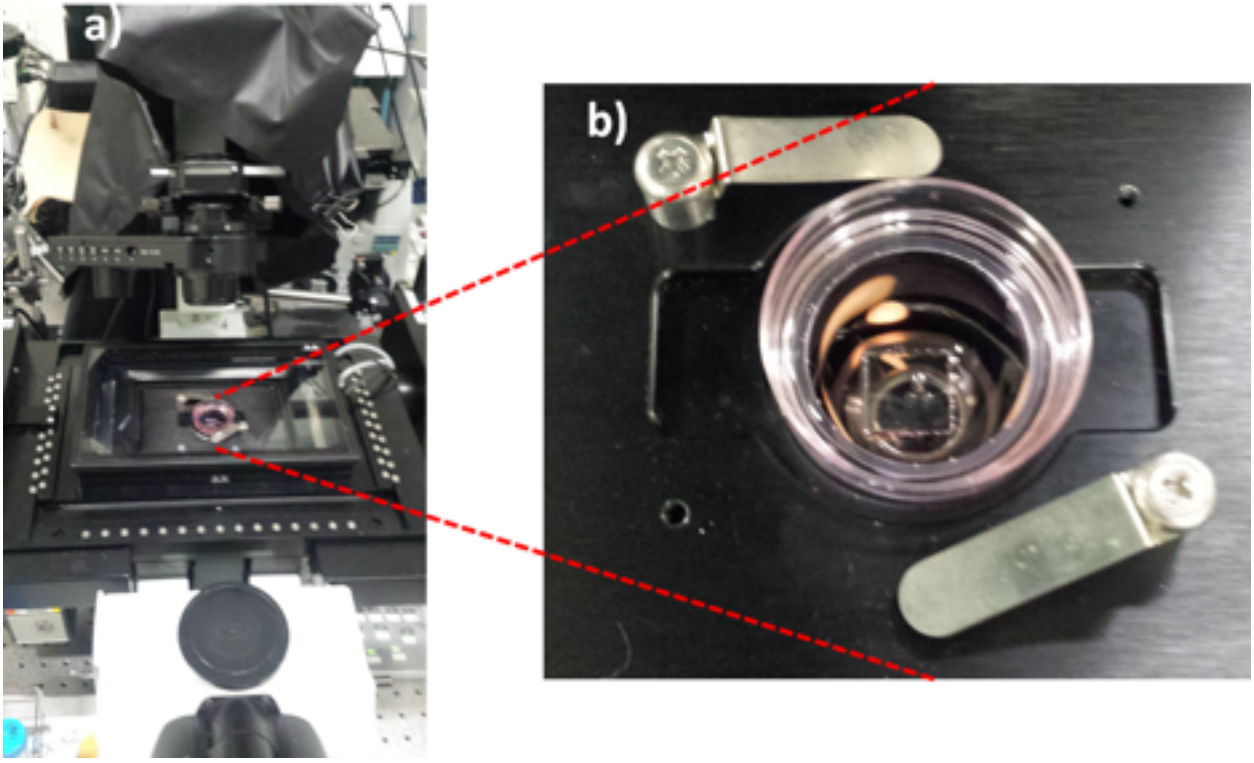

Figure S6*: Photos of a) the mini-incubator mounted on the inverted microscope system and b) a biochip inside a Petri dish.*