BioCombs4Nanofibers

Project BioCombs4Nanofibers

D2.9 Report on in-chip fabrication

Reporting period	from	**01.10.2020**	to	**30.09.2022**
Report completed and released		**29.03.2022** **Mathias Geiger**		

1. Goals and Detailed Description

Overall goal: **D2.9 Report on in-chip fabrication** is a Public report on the web-site on microfluidic in-chip fabrication of nanofibers. It will refer to a scientific article on these results.

Manmade chemical reactions are mostly performed on a large scale; in reactors the size of houses and chemical plants the size of small towns. In contrast, biological reactions happen at the small scale; inside organs, vessels and single cells. This small scale not only reduces the amount of reagents needed, but allows much more intricate and complex reactions. At small length scales, fluid flows in a laminar manner, along smooth paths without lateral mixing. This flow condition allows predicting and controlling reactant concentrations, bringing different reactants into controlled contact, and thus facilitates the complex reactions often found in biological systems.

Microfluidic systems are manmade chemical reactors that work on length scales comparable to biological systems. Reactions are performed inside channels smaller than a human hair. Microfluidic systems are used in many applications to reduce the required sample amount, such as point of care diagnosis or drug delivery. However, these systems also exhibit the same flow conditions like biological reactors and are therefore well suited to mimic biological processes.

One such process is the spinning of silk by various insects and spiders. This process leverages the flow conditions in small systems to assemble proteins into fibers, in a highly controlled and efficient manner. We try to mimic biological silk spinning and establish a technical process using microfluidic systems.

Microfluidic spinning uses a liquid spinning dope, containing a dissolved protein or polymer precursor. This spinning dope is focused into a shear fluid containing a non-solvent, to form a stable jet. Due to the laminar flow inside the microfluidic system, no convective mixing occurs

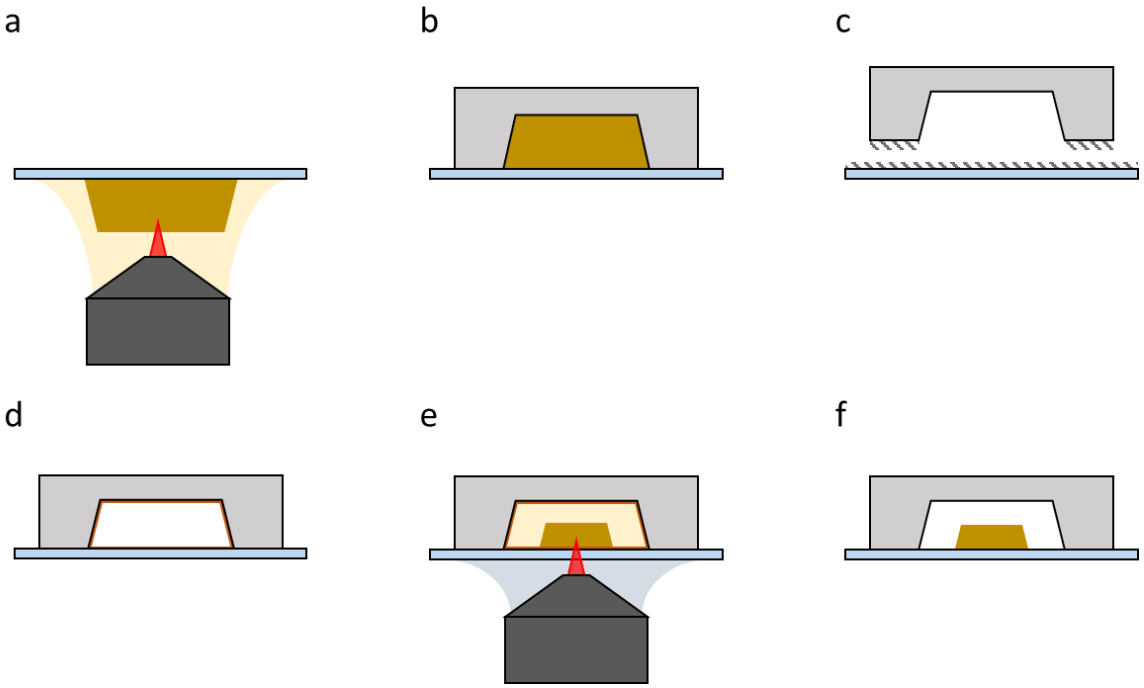

Fig. 1: Schematic representation of the fabrication process of microfluidic chips. **a** The master mold is 3D-printed with a 2-photon polymerization printer. **b** The master mold is replicated in polydimethylsiloxane. **c** The open channel is closed off with a glass slide by bonding with an oxygen plasma. **d** The channel is coated with TEOS:MTS to ensure good adhesion of the print to the channel. **e** The channel is filled with liquid photoresin and the structure is printed directly into the channel. **f** The remaining liquid photoresin is removed to yield the final structure. [1]

between the jet and the surrounding shear fluid. Over the length of the channel, the solvent and non-solvent from spinning dope and shear fluid, respectively, exchange due to diffusion. At a point, the dissolved protein or polymer precursor reaches the critical concentration, the spinning dope jet precipitates into a solid fiber and can be collected at the outlet of the microfluidic system.

A key condition for microfluidic spinning is the microfluidic system itself. The channels used have diameters between 50 and 100µm – the size of a human hair. These channels are designed with computer aided design tools and fabricated to fit the specifications down to feature sizes of 1µm. The channels are fabricated with soft lithography followed by in-chip direct laser writing to imprint a 3D-nozzle directly into the channel.

Soft lithography is an established process to produce microfluidic systems. In this process, a negative of the channel is taken as a master mold. This mold is replicated in an elastomer that is directly cured on the master mold. In most cases, polydimethylsiloxane (PDMS), a transparent, biocompatible elastomer, is used. After curing, the chip is peeled off the master mold, so that it carries the channel layout on one side. To close off the channels, a glass slide is bonded to the PDMS chip with an oxygen plasma. A schematic representation of the soft lithography process is shown in Figure 1 a-c.

The negative master mold is an integral part of the soft lithography process, and the available channel geometries are limited by the fabrication capabilities of master molds and their design

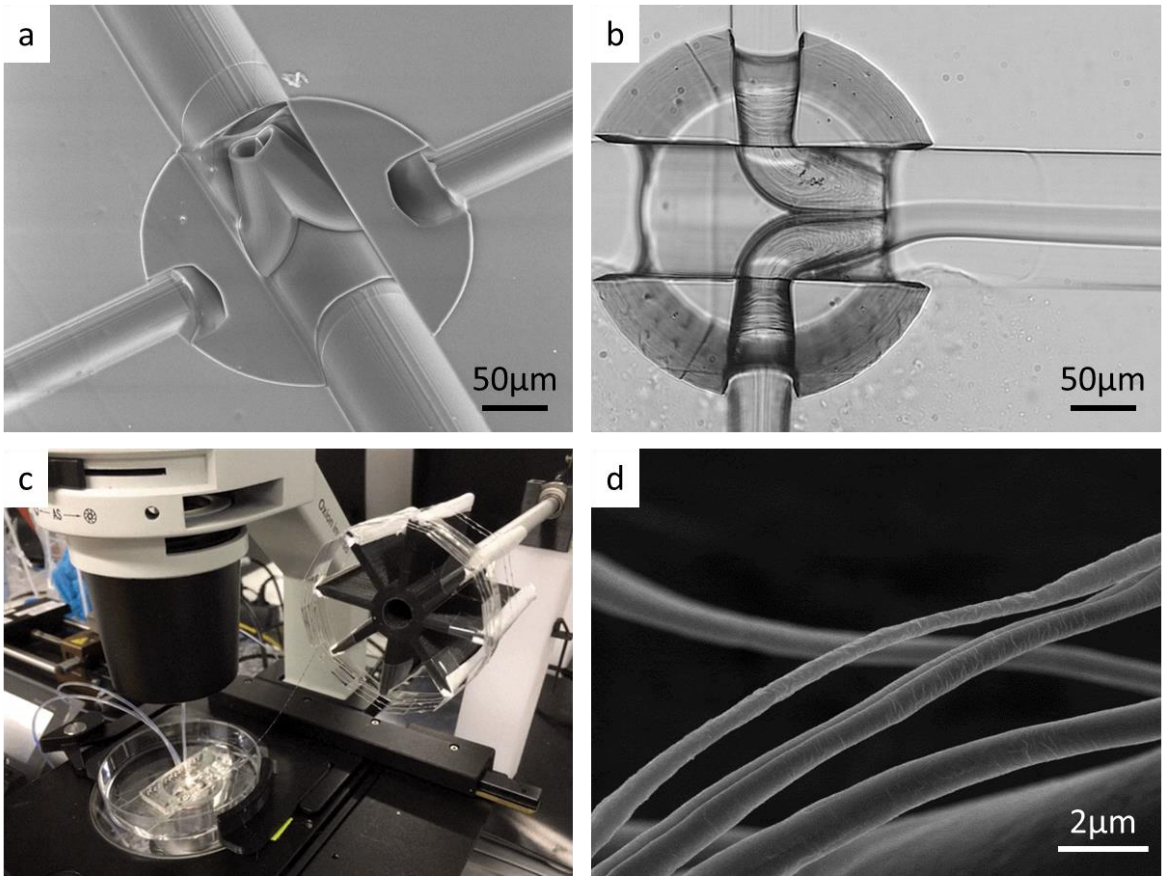

Fig. 2: a Electron micrograph of an in-chip direct laser written nozzle**. b** Brightfield micrograph of the microfluidic spinning process. **c** Photograph of the microfluidic spinning process. **d** Electron micrograph of as spun regenerated silk microfibers. [1]

constraints. We fabricate our master molds by 3D-printing with a 2-photon polymerisation printer; the nanoscribe professional gt+. This printer uses a near infrared laser to selectively polymerize a liquid photoresin, with a minimal lateral resolution of 1µm and a longitudinal resolution of 3µm.

Even though master molds can be produced with great design freedom and high resolution, the replication process limits the complexity of the final microfluidic chip. Soft lithography is a casting process, so undercuts or hollow structures can not be produced. In microfluidic spinning, we aim to focus our spinning dope into a jet, which requires a nozzle containing both undercuts and hollow structures. Therefore, we 3D-print a nozzle into the microfluidic chip, using in-situ direct laser writing.

For in-situ direct laser writing, the channels of the microfluidic chip are filled with a low viscosity liquid photoresist. The nozzle is printed with the nanoscribe professional gt+ directly into the microfluidic chip. After the print, the photoresist is flushed out from the channel, to leave only the polymerized structure. For better adhesion of the printed structure to the PDMS channel, the channel's surface is activated with an adhesive coating. A schematic representation of the in-chip direct laser writing is shown in Figure 1 d-f. A detailed description of the method can be found in [1, 2].

The combination of soft lithography and in-chip direct laser writing allows the fabrication of complex microfluidic chips with intricate functionalities. We produced different types of nozzles;

single nozzles, double nozzles for the production of multiple fibers in a single channel, twin nozzles for the production of twin fibers, structured nozzles for non-circular fibers.
We produced fibers from a wide range of materials, including polyacrylonitrile, alginate, and regenerated silk. The spinning process allows fine tuning of the diameter. It produces endless, individual fibers that can be collected and used further. In contrast to electrospinning, the fibers are thicker, but individual fibers can be collected and assembled into highly aligned meshes. Fibers produced with microfluidic spinning have been shown to be biocompatible and are a promising material for tissue engineering scaffolds, especially for highly oriented tissues such as cartilage, muscle and nerve tissue.
Other works on in-chip direct laser writing report printing of fluidic valves that control flow direction [3] and mixers [4]. Moreover, this technique has been adapted for the continuous production of complex shaped particles [5].

Literature:

[1] Lüken, A., Geiger, M., Steinbeck, L., Joel, A., Lampert, A., Linkhorst, J., & Wessling, M. (2021). Biocompatible Micron-Scale Silk Fibers Fabricated by Microfluidic Wet Spinning. Advanced Healthcare Materials, 2100898. **https://doi.org/10.1002/adhm.202100898**

[2] Lölsberg, J., Linkhorst, J., Cinar, A., Jans, A., Kuehne, A. J. C., & Wessling, M. (2018). 3D nanofabrication inside rapid prototyped microfluidic channels showcased by wet spinning of single micrometre fibres. Lab on a Chip, 18(9), 1341–1348. **https://doi.org/10.1039/C7LC01366C**

[3] Lamont, A. C., Alsharhan, A. T., & Sochol, R. D. (2019). Geometric Determinants of In-Situ Direct Laser Writing. Scientific Reports, 9(1), 394. **https://doi.org/10.1038/s41598-018-36727-z**

[4] Oellers, M., Lucklum, F. & Vellekoop, M.J. On-chip mixing of liquids with swap structures written by two-photon polymerization. Microfluid Nanofluid 24, 4 (2020). **https://doi.org/10.1007/s10404-019-2309-8**

[5] Lüken, A., Stüwe, L., Rauer, S. B., Oelker, J., Linkhorst, J., & Wessling, M. (2022). Fabrication, Flow Assembly, and Permeation of Microscopic Any-Shape Particles. Small, 2107508. **https://doi.org/10.1002/smll.202107508**

2. Evaluation of Goals and Resulting Actions

This report has been finalized and submitted in time in m30. It will be published on the website of the **BioCombs4Nanofibers** project (http://biocombs4nanofibers.eu). A reference to this report will also be published on Twitter and ResearchGate as project update for our followers.

Some results presented in this report are already published (Lüken, A., Geiger, M., Steinbeck, L., Joel, A., Lampert, A., Linkhorst, J., & Wessling, M. (2021). Biocompatible Micron-Scale Silk

Fibers Fabricated by Microfluidic Wet Spinning. Advanced Healthcare Materials, 2100898). Further results are planned to be published in scientific articles in the journals *Acta Biomaterialia* and *Small*.

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