FLOW OF NANOFIBRES SUSPENSION IN A MICROCHANNEL

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Abstract

Pressure driven and electrophoretic flows of fluorescent nanofibre filaments in a microchannel were observed and discussed. Behavior of filaments in the flow was then compared with theoretical predictions and models. The experimental results can be useful to validate numerical simulations of suspended micro and nano filaments in a microchannel flow.

1. Introduction

Recent developments of the lab-on-a-chip technology provide possibility to use microfluidic systems for manipulating, sorting and analysing individual biomolecules. Devices consisting of nanoscale pores are being pursued as probes of molecular structure **[1]**. Single, double or triple DNA strands passing nanopores are observed to extract the identity of DNA bases **[2]**. In such devices, molecular migration in flowing dilute polymeric solutions is a well-known phenomenon. The chain deformation and migration away from the confining walls greatly affect its transport dynamics **[3]**. However, rapid measurements of DNA fragment size, of their folding and unfolding sequences, still represent challenging target for present single molecule studies. Development and availability of highly sensitive experimental tools permits to follow the transient behavior of single macromolecules in a nearly native environment but such studies are still very difficult and expensive. The available experimental data are mostly quantitative, not accurate enough to be used for validating numerical models.

Since there is a common agreement that understanding slender filament dynamics in Stokes flow may elucidate fundamental questions about mobility and deformation of biological macromolecules due to hydrodynamic stresses from the surrounding fluid motion, the main goal of the work is to create experimental model to support and then verify numerical simulations of suspended micro and nano filaments in a confined microchannel flow.

In the following we present our preliminary examinated study, demonstrating behavior of short nanofibres suspension in a pressure driven and electrophoretic flow.

2. Materials and methods

2.1. Materials

The commercial material used to produce nanofibres was polylactic acid (PLA, Biomer® L9000, Biomer) of molecular weight 200 kDa solved in a mixture of chloroform (Chempur) and dimethylformamide (DMF, Chempur). All commercial compounds and solvents were of analytical purity and used as received without further purification. To prepare polymer solution (10% wt) suitable for electrospinning, PLA was dissolved in chloroform. The solution was heated for 5 min at 50^{o}C in a closed vial to speed up the dissolution process and then cooled to room temperature. Then DMF solvent was added and all components were well mixed by vortexing to obtain homogeneous solution. The chloroform to DMF mass ratio was 15:1. Finally, a small amount of rhodamine was added to the polymer solution before electrospinning to produce filaments visible under a fluorescence microscope.

2.2. Equipment

A custom-made high voltage power supply was the core of the electrospinning setup. It permits remote voltage adjustment in the range 0-30 kV, with a maximum current output of 0.3 mA. A sensitive (nanoampere range) amplifier was used to measure electric current carried by electrospun nanofibres. A constant volume flow rate of the polymer solution was maintained using a syringe pump (New Era Pump Systems, NE-1000). A high speed CMOS camera (PCO Imaging, pco.1200hs) was used to observe and record the electrospinning process. An epifluorescence microscope (Nikon, Eclipse E-50i) supplied with a high speed CMOS camera (PCO Imaging, pco.1200hs), high pressure mercury lamp (Nikon, LHM 100C1) and FITC fluorescence filters (excitation band 465-490 nm, emission band 515-555 nm) were used to characterize optical characteristics of nanofibres and to evaluate their fluorescence. The pressure driven flow was performed with use of the syringe pump (New Era Pump Systems, NE-1000), epifluorescence microscope (Nikon, Eclipse E-50i) and a high speed camera (PCO Imaging, pco.1200hs). The electrophoresis was performed with use of electrophoresis power supply (Convert, EV262), epifluorescence microscope (Nikon, Eclipse E-50i) and a high speed camera (PCO Imaging, pco.1200hs). Leica CM1850 cryostat was used to cut electrospun fibres to short filaments.

2.3. Electrospinning process

Micro filaments used in the experiment were produced by electrospinning the polymer solution. 10% wt solution of PLA was used to form short, insoluble in water

and fluorescent filaments. To obtain controlled deposition of PLA fibres, a rotating drum, covered with aluminium foil was used as a target. Producing possibly most parallel deposited fibres is was very important in further fibres cutting process. As a spinneret, a needle with 4 mm length and 0,35 mm internal diameter was used. The spinneret-target distance was set to 15 cm. After several tests the optimal electrospinning parameters were assigned: 10 kV voltage applied and 800 ml/h flow rate. The experiment was conducted at room temperature and about 30% air humidity.

2.4 Fibres cutting process

A square cutaway of aluminium foil (2 cm x 2 cm) covered with electrospun PLA fibres was frozen to -21^{o}C and then cut by the cryostat apparatus to pieces of 30 μm width. The direction of cryostat blade motion was perpendicular to fibres arrangement direction in order to obtain short filaments of similar lengths. Because of some imprecision of the cryostat apparatus there was a spread of fibre filaments lengths which varied from 25 to 70 μm.

2.5 Nanofibres in microchannels

The flow of suspended nanofibres was studied in polycarbonate channels, in channels created in a PDMS mould and in a straight channel formed between two glass plates separated by a thin Teflon film. The flow was then illuminated and observed through the upper wall of the channel. Fluorescence of rhodamine suspended in filaments was excited by a high pressure mercury lamp and emitted red light (610nm) was collected using appropriate filters and the camera. By traversing the field of observation in the horizontal and vertical direction, the position of the interrogated flow plane was selected. For the recording of images a high speed camera was used. Fluorescent light from filaments suspended in the flow was collected by the camera at given time steps. Translocation of filaments within the channel and their shape evolution were evaluated using image processing software. To obtain quantitative data on the fibre shape we extracted and analyzed the fibre profile for each frame of the recorded movie and digitized data to fit the fibre shape to a polynomial. This polynomial was used to generate predefine initial configuration for the numerical simulations.

2.6 Pressure driven flow

The flow was studied in a straight channel formed between two glass plates separated by a thin Teflon film. The channel width was 2 mm. Suspension of polymer filaments in water was inserted to the channel through a Teflon pump tube with use of a plastic 1 mL syringe fitted with a needle of 0,5 mm inner diameter. The pressure driven flow was provided by a syringe pump. The behaviour of filaments in a pressure driven flow was observed and then compared with predictions of the Stokesian bead-spring model for dynamic behaviour of the fibre suspended in a Poiseuille flow between two infinite parallel walls **[4]**.

2.7 Electrophoretic flow

The separation of spieces by electrophoresis in a thin channel is dependent on the differential migration of analytes in an applied electric field. The spieces separate, as they migrate, due to their electrophoretic mobility. The electrophoretic mobility is proportional to the ionic charge of a sample and inversely proportional to any frictional forces present in the buffer. When two species in a sample have different charges or experience different frictional forces, they will separate from one another as they migrate through a buffer solution. The frictional forces experienced by a spiece depend on the viscosity of the medium and the size and shape of the charged spiece. That is why smaller spieces will migrate faster than those bigger ones. The velocity of migration of an analyte in microchannel electrophoresis will also depend upon the rate of electroosmotic flow of the buffer solution. The electroosmotic flow is directed toward the negatively charged cathode so that all spieces, positively or negatively charged, are pulled through the capillary in the same direction by electroosmotic flow of the buffer solution. The electroosmotic flow results in a flat velocity flow profile so that velocity of spieces moving close to the channel walls cannot be representatively compared with the velocity of those in the channel centre. That is why in our experiment the filament flow velocity was in all cases measured in the centre of the channel.

The flow was studied in a polycarbonate microchannel of rectangular cross section. Channel dimensions were: 1200 μm width, 1200 μm depth and 5 cm length. Suspension of polymer filaments in TBE buffer (a buffer solution containing a mixture of Tris base, boric acid and EDTA, commonly used for electrophoresis) was inserted to the channel through a Teflon pump tube with use of a plastic 1 mL syringe fitted with a needle of 0,5 mm inner diameter. The behavior of fibre filaments in the electrophoretic flow was observed and filaments velocity on the supplied voltage was measured.

3. Results and discussion

During the electrospinning process PLA fibres of 1 μm diameter were obtained **(Figure 1)**. After fibres cutting process about 40 μm long PLA filaments were obtained (**Figure 2**).

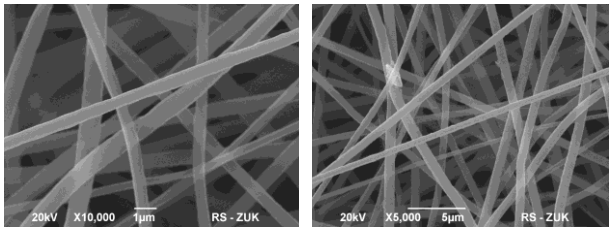

Figure 1: *Electrospun PLA fibres before cutting process (SEM photos). Fibre diameters varied between 0,7 μm and 1,2 μm.*

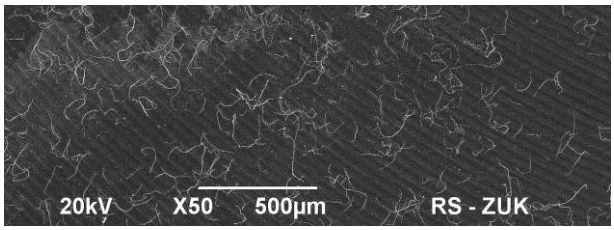

Figure 2: Electrospun PLA fibres after cutting process (SEM photo). Fibre lengths varied between 25 µm and 70 µm.

3.1 Dynamics of nanofibres in a microchannel flow.

3.1.1 Dynamics of PLA fibre filaments in the pressure driven flow.

Steady and pulsating flow was generated to analyze behaviour of suspended nanofibres. In most cases, after single nanofibre was localized in viewing area of the camera, the flow was stopped and reversed. Such push-pull motion permitted to observe selected object without changing position of the channel under the microscope. It was found that in our experiments fibres conveyed through the straight channel are usually already deformed by the initial flow shear stresses (**Figure 3**). These deformations appear to remain almost unchanged for the level of shear stresses generated by the flow. Tumbling, rotating type of fibre motion was observed but no filament shape changing during the flow was noticed. The sequence of images is extracted from the longer movie and illustrates dynamics of the nanofibre fragment located about 40*µm* from the channel wall.

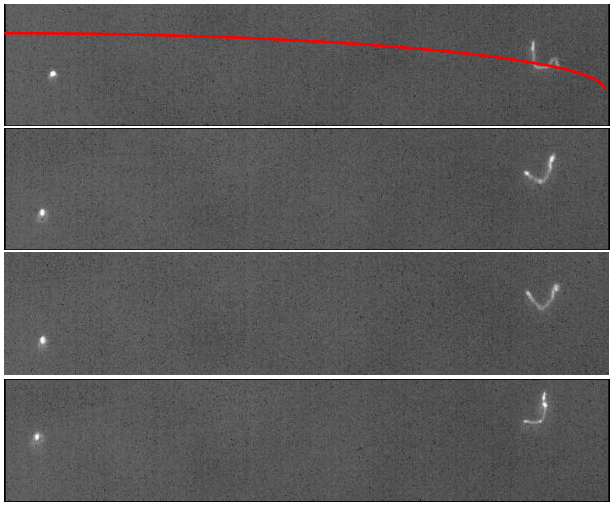

Figure 3: *A sequence of images of a nanofibre conveyed by a pressure driven flow. The microchannel width is 2 mm and the mean flow velocity is 40 µm/s. Channel axis is on the left side and less than one half of the channel is visible. Poiseuille velocity profile is indicated in the top image. The bright spot on the left is a fluorescent particle used to measure local flow velocity.*

Figure 4 shows comparison between experimental and theoretical results **[4]**. Our results are inconsistent with theoretical predictions, where filaments change their conformation during the flow. Much bigger filament flexibility assumed for theoretical calculations may be the reason of such incompatibility.

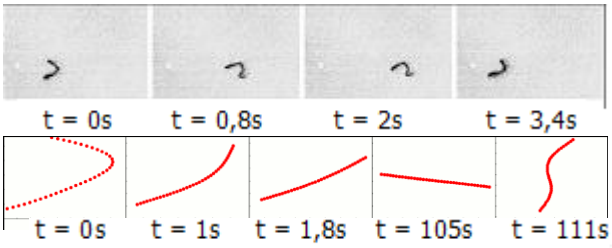

Figure 5: *Fibre filaments behaviour in pressure driven flow: experimental results (top) in comparison with numerical folding fibre model (bottom)* ***[4]****. Relative time (t) is given under the images.*

Top images in **Figure 4** are extracted from the experiment for a PLA fibre filament in a pressure driven flow. Fibre rotates counter-clockwise. Flow direction is from left to right. The initially C-like fibre keeps its shape almost unchanged. Visible shape changes are probably due to the optical projection. Bottom images are from numerical folding fibre model. Fibre rotates clockwise. Flow is from left to right. The initially C-like fibre aligns with the flow and then, after a long time, it quickly flips and remains elongated again. During the flipping, when the fibre is oriented across the flow, it becomes slightly deformed, however its shape does not coincide with the starting condition. Flipping period is approximately 115*s*.

3.1.2 Dynamics of PLA fibre filaments in the electrophoretic flow.

Initial observations of fibre filaments a electrophoretic microchannel flow indicated, as well as in the pressure driven flow, indicate that PLA filaments act as solid and do not change their shape during the electrophoretic flow. Straight fibre filaments orientate and flow according to the channel axis (**Figure 5**), curved filaments also preserve their original shape **(Figure 6**).

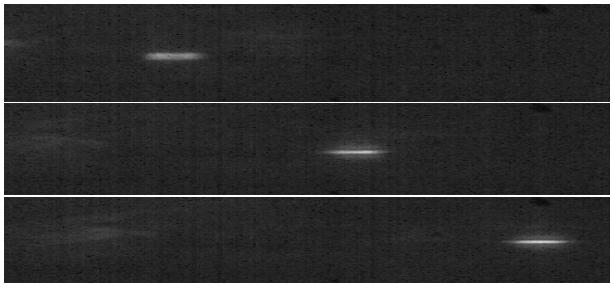

Figure 5: *Straight PLA fibre filament in the electrophoretic flow. Fibre length is 35 µm. Fibre width is 1 µm. The microchannel width is 1.2 mm and the fibre flow velocity is 9.5 µm/s. Flow direction is from left to right. Images from PCO high speed camera were obtained for 450 V electrophoretic voltage.*

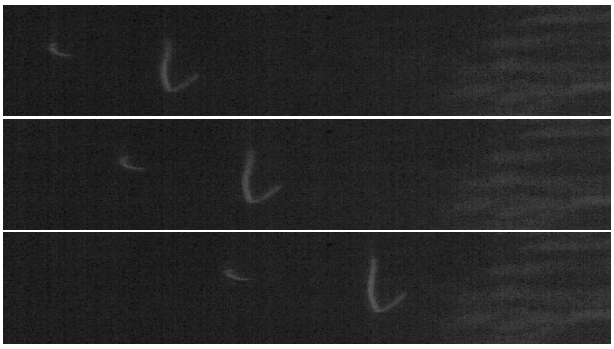

Figure 6: *C-shaped PLA fibre filament in the electrophoretic flow. Fibre length is 60 μm. Fibre width is 1 μm. The microchannel width is 1.2 mm and the fibre flow velocity is 5.5 μm/s. Flow direction is from left to right. Images from PCO high speed camera were obtained for 300 V electrophoretic voltage.*

The migration of the nanofibres is initiated by the electric field that is applied between the inlet and the outlet of the channel. It is important to note that all liquid ions, positive or negative, are pulled through the channel capillary in the same direction by electroosmotic flow. The fibres migrate due to their electrophoretic mobility and they interact with plug velocity profile of the electroosmotic flow. Hence, in a straight channel there are no mechanisms for fibre deformation or rotation. This is confirmed by **Figures 5** and **6**. Hence, the electrophoretic migration velocity can be used to indicate information about a fibre length or its shape. These can be used to separate fibres of desired length or shape for further investigations.

Here, straight polymer filaments of 40 μm length and 1 μm diameter, moving along the channel axis were selected to analyse the relationship between electric filed strength (voltage) and the filaments flow velocity. The result is shown in **Figure 7** and **Table 1**. As predicted, the results indicate increased fibre filaments velocity with the increased electric potential.

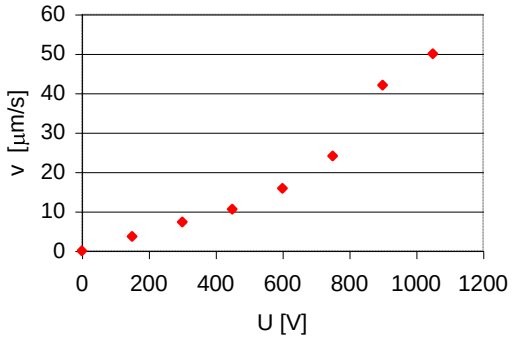

Figure 7: *Effect of electric potential on fibre electrophoretic velocity (channel width 1,2mm, fibre length 40 μm, fibre diameter 1 μm).*

Table 1: *Effect of electric voltage on fibre electrophoretic velocity (data of Fig. 7).*

U [V]	0	150	300	450	600	750	900	1050
V [μm/s]	0	3,65	7,31	10,58	15,87	24,04	42,02	50,00

The relationship between fibre filaments flow velocity and fibres length was also measured. Three different, straight fibre filaments of 34, 49 and 57 μm length were observed and then their velocities were compared. Results shown in **Table 2** indicate the decreasing fibre filament velocity with the increase of fibre filament length.

Table 2: *Fibre migration velocity as a function of fibre length; (electric voltage 300V, channel width 1,2mm, fibres diameter 1 μm).*

L [μm]	v [μm/s]
34	9,62
49	8,41
57	6,61

These results can be useful in further investigations in order to find the best method of selecting fibre filaments of desired length. In case of DNA fragments studies and for creating experimental model of their behaviour in a native environment, such as passing the nanopores or folding and unfolding sequences, it is very important to be able to obtain fibre filaments of controlled length and diameters. As one can see, the mechanical methods used for cutting the fibres give imprecise results so further research of electrophoretic fibre filaments length distribution will be continued.

4. Conclusions

To meet expectations of numerical models used for simulating DNA and protein bio flows further experiments are necessary to optimize electrospinning process in order to obtain thinner and more flexible fibres. Larger ratio of fibre length to diameter should provide higher fibre flexibility. Such fibres will be better suited to model objects like DNA or proteins chains. The authors also intend to study electrophoretic flows for different fibre shapes (straight, semicircular, s-shaped) in order to compare experimental result with the theoretical predictions used to calculate the effective Stokes diameter of molecules with complex shape **[5]**.

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