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FLUID DYNAMICS OF BIODROPLETS UNDER ELECTROSTATIC ACTUATION

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ABSTRACT: *The present work addresses the development of a microfluidic chip to perform biochemical and clinical analysis. The biosamples are transported inside microdroplets, which move on an open configuration chip, using interdigited electrodes, by electrostatic actuation. The results consider different chip configurations and the discussion addresses the effect of the wettability on the fluid dynamics of the biodroplets. The results clearly show that the chip configuration to be selected is governed by the fluid dynamics of the droplets, namely the temporal evolution of the spreading diameter and the velocity of the moving droplet. The microfluidic devices are more efficient in the transport of protein solutions than of cell suspensions. This is attributed to cell deposition and migration processes, which affect droplet motion.*

1 INTRODUCTION

Lab-on-chips are microfluidic devices capable of performing several programmed operations and biochemical analysis, which would otherwise require the use of multiple expensive and complex laboratory equipment. Many of these devices are closed configuration systems based on the flow of the biofluids inside microchannels. However, there are several difficulties in their implementation related to their geometric complexity, to the need of auxiliary pumping equipment, clogging and difficulties in assessing the samples. Alternatively, the transport of the biosamples in small droplets on open configuration chips, controlled by electrostatic actuation has shown great potential for lab-on-a-chip applications [1]. Despite a few authors report the successful transport of physiological fluids on open configuration systems e.g. [2-3], sparse information is still reported in the literature concerning the transport of biofluids (exception made for instance to [4]), so that it is still not clear which are the most suitable electrochemical properties of the

fluids or the most important parameters governing biofluids transport and manipulation. The properties of the dielectric substrate are also sparsely discussed in the literature, although they play a vital role on controlling droplet motion. For instance, the substrates should by lyophobic or superlyophobic to the biofluids in study, but hysteresis caused by contamination and/or surface topography must also be addressed, as it may lead to asymmetries and instabilities in droplet shape and actually preclude droplet motion due to the large energy dissipation at the contact line between the droplet of the biofluid and the substrate [4-5]. Contamination of the substrates is often promoted by the passive adsorption of the biocomponents. Although this problem has been recognized by different authors, e.g. [6], its effects on the surface wettability and consequently on the sample transport have been taken to a secondary level. However, [4] have recently shown that adsorption locally reduces the contact angle, which aids droplet spreading but also promotes energy dissipation at the contact line, thus precluding droplet receding and making the droplet transport more difficult. Hence, this effect must be considered for the selection of the materials during the design and configuration of the microfluidic chips. Finally, there are numerous papers concerning electrowetting applications on microchips (including lab-on-chip systems), but very little information is provided regarding the chip configuration. As the droplet is transported on the chip dielectric surface, the size and distance between electrodes must account for the wetting properties and how they affect droplet dynamics, which should be balanced by the particular parameters affecting the electrical field.

Within this scope, the work presented here addresses the dynamic behaviour of biofluid droplets under electrostatic actuation, on an open configuration system, using co-planar interdigited electrodes. Given that the droplet motion must be controlled by the programmed actuation on the co-planar electrodes, to assure the generation of an electrical force in the desired droplet motion, instead of performing a very complex programming of the microchip, an alternative approach was considered here which consists on imposing an adjustable frequency to the electrical signal. A similar solution was implemented for instance by [7]. A detailed analysis on the wetting properties of several dielectric substrates is performed in the first part of the work, which details the design and configuration of the test microfluidic chips. Afterwards, the results focus on the effects of the wetting and physico-chemical properties of the substrates and of the biofluids on droplet motion. The analysis, based on the spreading diameters and droplet displacement velocity at the contact line, determined for different experimental configurations, aims at determining the most favourable chip configuration allowing an effective transport and manipulation of the biofluids studied here.

2 MATERIALS AND METHODS

The assays characterize the dynamic behaviour of biofluid droplets transported on open microfluidic chips, with different configurations (size of the electrodes and distance between them) and diverse wetting properties of the dielectric substrate. The fluids used are Green Fluorescent Protein (GFP) solutions and GFP-expressing *Escherichia coli* cell suspensions, with different concentrations, namely 1.71×10^{-3} mM for the GFP solution and 1×10^{9} cells/mL and 2×10^{9} cells/mL for the *E-Coli* solutions.

2.1 EXPERIMENTAL SET-UP

All assays were performed inside a Perspex chamber with total dimensions of 55x80x90 mm^3. This chamber has quartz windows to avoid optical distortion, which introduces

errors in the image based techniques. The chamber was saturated with the working fluid and the tests were performed under continuous monitoring of temperature and relative humidity of the surrounding air.

The various chip configurations tested here were modelled in SOLIDWORKS and converted to AutoCAD files to be manufactured at INESC-MN. They were then printed on a 0.6 μm aluminium film by lithography on a glass substrate with 102x45 mm^2 and 700 μm thick. Finally, a thin film of a dielectric material was deposited on the chip assembly, without covering the contacts. The different configurations tested only vary in the electrodes width, *w* (between 120 and 1400μm), being the numerous interdigitate electrodes displaced with a fixed distance between them, 2a=60μm. The length of the electrodes is 24mm. Since the basic principle for droplet motion in microchips requires switching polarities between neighbouring electrodes, within an imposed frequency and following a similar approach explored by [7], a custom made frequency was imposed to the chips. This frequency could be varied between 50Hz and 400Hz, within 50 Hz increments. The applied voltage was varied from 0 to 245VDC, using a Sorensen DCR600-.75B power supply.

The solutions of GFP and *E-Coli* were characterized in terms of density, viscosity and surface tension, as in [4]. Tab. 1 summarizes the physico-chemical properties of the fluids used in the present work.

Tab. 1 Thermo-physical properties of the working fluids.

Solution	Density, ρ [g/cm^3]	Surface tension, σ_{lv} [mN/m]
Distilled water	0.998	72.2±0.7
GFP (1.71x10^{-3} mM)	0.990	73.80±0.04
E-coli (1x10^9 cells/ml)	0.991	71.793±0.325
E-coli (2x10^9 cells/ml)	0.982	61.327±0.203

The table shows that being aqueous solutions, the properties of the biofluids tested here are close to those of water, also presented in the table, which is taken as a reference fluid. Therefore, the dynamic viscosity of the solutions, all of them Newtonian, in terms of their rheological characteristics, was considered constant and equal to that of water, i.e. 1.0x10^{-3} Ns/(m^2) [8].

2.2 EXPERIMENTAL METHODS

The wetting properties of the dielectric substrates should play a major role on the dynamic response of the droplet. Hence, various dielectric materials were tested and characterized in terms of topography and wettability. The topography was quantified using a Dektak 3 profile meter (Veeco) with a vertical resolution of 20nm. Since all measured values are within this resolution, the roughness of the surfaces is negligible. The wettability was quantified for the different biofluids by the static contact angle and by the hysteresis, determined as the difference between the quasi-static contact angles (advancing and receding) measured with the optical tensiometer THETA from Attention. These measurements were performed as described in [4].

The dynamic response of the droplets is discussed based on the evaluation of the temporal evolution of the droplet-surface contact diameter and of the velocity of displacement of the contact line. These quantities were determined based on a home-made post-processing routine developed in Matlab applied to high-speed images, taken at 2200 fps using a Phantom v4.2 from Vision Research Inc., with 512x512 pixels@2100fps resolution. For the present optical configuration, the spatial resolution was 25 pixel/μm and the temporal resolution was 0.45ms. Temporal evolution of the contact diameter is presented as the average curve of at least 3 events, obtained at similar experimental conditions. Accuracy of the measurements is evaluated to be ±25μm.

To infer on the possible adsorption of the biocomponents on the dielectric substrates, simple tests were performed in which droplets of the biofluids (with different concentrations) were deposited on the surfaces. Afterwards, a sequence of tests with and without electrostatic actuation was performed and the droplets "footprints" over the substrates were observed on a Laser Scanning Confocal Microscope (Leica SP8). The images were taken with a 4X magnification (0.10 of numerical aperture), with a pixel size of 5.42 μm x5.42 μm. The obtained images were then post-processed on ImageJ, to determine the mean grey intensity (sum of intensities divided by the number of pixels in the region of interest of the droplet footprint) and the Area Integrated Intensity (sum of intensities of pixels in the region of interest of the droplet footprint normalized by unit of area). To reduce the noise, the average grey intensity levels of the background image were also subtracted. The final result is the Total Corrected Droplet Fluorescence (TCDF), as proposed by [4]. Higher values of TCDF are associated to a larger quantity of the protein or cells adsorbed by the substrate.

3 RESULTS AND DISCUSSION

3.1 WETTING PROPERTIES OF THE DIELECTRIC SUBSTRATES

The study of the wetting properties is very important, concerning the selection of the most appropriate dielectric material to use when manufacturing the chips, in order to reduce the costs and improve the efficacy in the design of the test chips.Tab. 2 depicts the equilibrium angles, obtained for each fluid tested, on the various dielectric coatings tested in the present study. The values of the contact angles are very similar for every fluid, being the differences attributed to the experimental variations associated to the hysteresis and small chemical surface heterogeneities.

Tab. 2 Equilibrium contact angles, obtained for each pair fluid-dielectric substrate considered in the present work.

Dielectric coating	Contact angle [°]		
	Water	*E-coli*	GFP
Teflon	112±5	103±6	121±6
Teflon with Glaco	145±1	141±9	153±3
PDMS	121±1	112±1	119.5±0.4
PDMS with Glaco	153±3	153±2	155±3
SU8 *resist*	67.1±0.7	65±2	71.8±0.2
SU8 with Glaco	160±7	162±1	153±4
Si_3N_4	64.1±0.7	59±4	65±2

The wetting behaviour of the substrates is not completely characterized solely using the static contact angles, as they do not provide any information, for instance on the possible energy dissipation within the contact line between the drop and the surface, during spreading. Thus, an analysis of the contact angle hysteresis was additionally performed, as depicted in Fig. 1 for distilled water, GFP solution ($1.71x10^{-3}$ mM) and *E-coli* suspensions ($1x10^{9}$ cells/mL). The results gathered in Tab. 2 and in Fig. 1 show that only the SU8 resist and Si_3N_4 surfaces are hydrophilic, being the others hydrophobic. The highest contact angle of 121º is obtained for the PDMS substrate. Despite having high contact angles, which is desired for the current application in order to endorse droplet motion, PDMS and Teflon substrates depict also high hysteresis, which in turn is proportional to the surface adhesion and promotes energy dissipation at the contact line. This dissipation is one of the main factors precluding the recoiling motion, as recently shown by [4]. In addition, [4] report that the GFP protein was adsorbed by the Teflon substrate, leading to a local increase of surface wettability and further contributing to preclude the receding motion, as this wettability increase is irreversible, taking the contact angles to values near saturation.

In this context, this work also inferred on the possible adsorption of the GFP and *E-coli* cells by the substrates, quantified by

the TCDF. The minimum values of TCDF = $8.88x10^{7}$and TCDF = $1.65x10^{7}$ were obtained for the adsorption of GFP ($1.71x10^{-3}$ mM) and *E-Coli* suspension ($1x10^{9}$ cells/mL) on PDMS, respectively. Larger values of TCDF were obtained for all the other substrates.

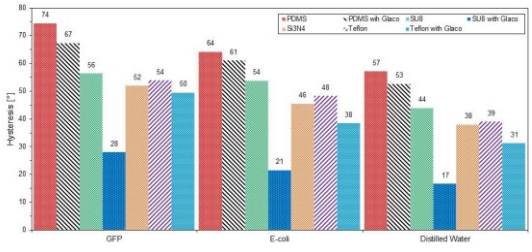


Fig. 1 Contact angle hysteresis evaluated for GFP solution, GFP-expressing *E-coli* suspension and distilled water on the tested dielectric substrates.

To further promote droplet motion, a commercial coating was used, named Glaco©, which is mainly a perfluoroalkyltrichlorosilane combined with perfluoropolyether carboxylic acid and a fluorinated solvent [9]. Fig 1 clearly shows a significant minimization of the hysteresis when the surfaces are coated with Glaco®. Additionally, the surfaces become superhydrophobic. The application of Glaco© coating is observed to further reduce the adsorption of both proteins and cells, decreasing the TCDF values in about one order of magnitude. These results are not shown here due to paper length constraints.

3.2 DYNAMIC RESPONSE OF THE BIOFLUID DROPLETS IN ELECTROWETTING

The spreading diameter of GFP and *E-Coli* suspension droplets, was evaluated under electrostatic actuation for the test chips coated with PDMS, SU8 resist and both materials further coated with Glaco®. As discussed in section 3.1, this coating reduces the adsorption of the proteins and of the cells by the substrates, so the irreversible contact angle decrease associated to the adsorption mechanisms is also minimized. Furthermore it decreases substantially the contact angle hysteresis. This modification of the wetting properties has a dramatic effect on the droplet transport on the chips, as shown in Fig. 2 which depicts the temporal evolution of the spreading diameter of a GFP droplet under electrostatic actuation, made non-dimensional with the initial diameter of the deposited droplet, i.e. for 0V ($d(t)/d_{0V}$). t=0ms corresponds to the beginning of the actuation. The figure shows a significantly larger spreading diameter with the SU8 resist coated with Glaco®, being followed by a pronounced recoiling, allowed by the noteworthy hysteresis reduction. The worse response of the droplet to the actuation obtained for PDMS coated with Glaco® is associated to the large final thickness of the substrate, which, according to Young-Lippmann equation, precludes the decrease of the contact angle under actuation. Despite this reduced response, evident recoil is still observed. The dynamic response of the droplets also depends on the properties of the biofluids.

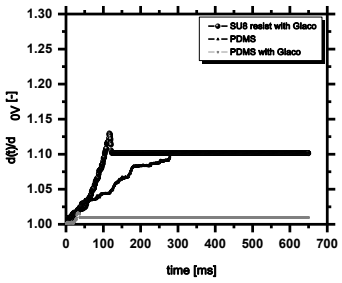
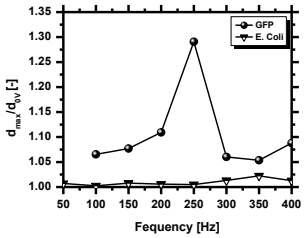
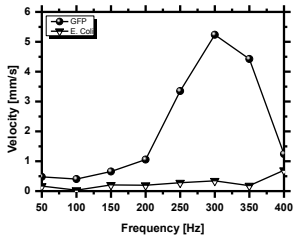


Fig. 2 Electrowetting induced spreading diameter of GFP droplets for different dielectric substrates, between coplanar electrodes for the configuration w=1200µm, at 230V and 350 Hz. Initial droplet diameter is 2.8mm.

Fig. 3 depicts the non-dimension maximum spreading diameter and displacement velocity of GFP and *E-Coli* suspension droplets on the chip with w=1200µm coated with PDMS, for 230V, as a function of the imposed frequency. The results depicted here were obtained for the lowest concentration of the *E-Coli* suspension, but similar trends were obtained for the highest.



a)



b)

Fig. 3 a) Maximum spreading dimensionless diameter and b) displacement velocity of GFP ($1.71x10^{-3}$ mM) and GFP-expressing *E-coli* suspension ($1x10^{9}$ cells/mL) droplets moving between coplanar electrodes (transport section of the device). Initial droplet diameter is 2.8mm.

The figure evidences the worse response of the cell suspension droplets, although the surface tension and density only vary slightly between the different solutions (see Tab 1). This behaviour is attributed to the large propensity of the cells to adhere to the surface and agglomerate near the contact line region (as observed on the confocal microscope when evaluating adsorption) which may be altering the local density and wettability. However, deeper studies must be performed to further understand these results.

4 CONCLUSIONS

The present paper addresses the dynamic response of biofluid droplets to electrostatic actuation, focusing on the role of the wetting properties of the substrate. The results show that the major obstacle to the droplet spreading is the energy dissipated at the contact line, associated to large hysteresis, which limits the spreading diameter, but also precludes the droplet recoiling. The results also evidence a non-negligible effect of the adsorption of the biocomponents on the substrates, which locally decreases the contact angle to values close to that of saturation, further limiting the droplet spreading. Protein suspensions are easier to transport than cells. This is attributed to the large propensity of the cells to adhere to the surface and agglomerate near the contact line region, thus altering the local density and wettability.

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