Universidade do Minho
Escola de Engenharia

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Dissertação de Mestrado
Ciclo de Estudos Integrados Conducentes ao
Grau de Mestre em Engenharia Biomédica

Trabalho efetuado sob a orientação do
Professor Doutor João Filipe Colardelle da Luz Mano

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DECLARAÇÃO

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ABSTRACT

For the last two decades, biomimetic superhydrophobic materials have been acquiring huge interest for distinct application fields such as textile and glass industries, biomedical *in vivo* devices and microfluidic systems. Different methodologies and materials have been employed to fabricate these substrates, prevailing in literature the use of complex processes with non biodegradable and costly materials.

In recent years, paper has emerged as a promising material for microfluidic and lab-on-chip devices, presenting interesting properties such as extreme availability, low-cost, flexibility and ability to be devised in different manners. Also the possibility to be functionalized and chemically modified allows researchers to exploit different techniques to pattern the paper surface, such as photolithography, plasma treatment and printing approaches. So far, the related literature is confined in the creation of hydrophobic-hydrophilic contrast and only a few works have reported the fabrication of superhydrophobic paper-based platforms.

Inspired by nature, the work presented in this thesis suggested the fabrication of paper substrates with water repellent properties to employ in a great range of possible applications. Biomimetic superhydrophobic paper surfaces were obtained with a simple phase separation methodology using poly(hydroxybutyrate). Scanning electron microscopy (SEM), water contact angle measurements and x-ray photoelectron spectroscopy (XPS) confirmed that, even presenting the same surface topography, the superhydrophobic surfaces differ in wettability from the original ones as a result of the modification of surface chemistry. Bi-dimensional superhydrophobic paper was used to develop open microfluidics and lab-on-paper devices, employing different patterning strategies; from equipment-dependent plasma treatment and UV/ozone irradiation to simple and extremely inexpensive writing processes, all are possible to generate more wettable domains onto rough paper surfaces. Additionally, benefiting of the paper properties we suggested the use of water resistant paper substrates to design essential non-planar labware for liquid manipulation, transport, mixing or storage. The results demonstrated that it is possible to obtain alternative and extremely low-cost lab apparatus, with lower propensity to adsorb proteins than original paper and that maintain superhydrophobic properties, even when subjected to ethylene oxide sterilization.

RESUMO

Durante as últimas décadas, os materiais biomiméticos com propriedades superhidrofóbicas têm vindo a adquirir um enorme interesse para aplicações, como as indústrias têxtil e do vidro, sistemas biomédicos implantáveis e de microfluídica. Para o fabrico destes substratos têm sido utilizados diferentes materiais e metodologias, prevalecendo ainda o uso de materiais não biodegradáveis e processos complexos e dispendiosos.

Nos últimos anos, o papel tem emergido como um material promissor para o desenvolvimento de dispositivos de microfluídica e de monitorização para testes biológicos/químicos, apresentando propriedades interessantes como extrema disponibilidade, baixo custo, flexibilidade e capacidade de ser utilizado de diferentes formas. A possibilidade de modificá-lo quimicamente permite aos investigadores utilizarem diferentes técnicas de modelação superficial, como fotolitografia, plasma e impressão. Até agora, a literatura está circunscrita à criação de um contraste hidrofóbico-hidrofílico e poucos trabalhos têm reportado a produção de papel superhidrofóbico.

Inspirado na natureza, este trabalho sugere o fabrico de substratos de papel com propriedades repelentes à água, para aplicar numa vasta gama de possibilidades. As superfícies de papel superhidrofóbicas foram obtidas por uma metodologia de separação de fases, usando polihidroxibutirato. Técnicas como microscopia eletrónica de varrimento (SEM), medição de ângulos de contacto e espectroscopia fotoeletrónica de raios X (XPS) confirmaram que, mesmo apresentando a mesma topografia superficial, os substratos rugosos diferem do papel original na molhabilidade, como resultado da modificação da composição química da superfície. O papel superhidrofóbico foi assim usado para criar dispositivos de microfluídica aberta e monitorização biológica/química, empregando diferentes estratégias de modelação superficial. Desde o tratamento por plasma ou a irradiação por UV/ozono até a simples processos de escrita, todos são passíveis de gerar domínios mais molháveis nas superfícies superhidrofóbicas. Adicionalmente, beneficiando das propriedades do papel, nós sugerimos a utilização dos substratos resistentes à água para construir material de laboratório para manipulação, transporte, mistura e armazenamento de líquidos. Foi possível obter uma alternativa extremamente económica para material laboratorial, com baixa propensão para adsorver proteínas, quando comparado com o papel original, e que mantém propriedades superhidrofóbicas mesmo quando sujeito a esterilização por óxido de etileno.

TABLE OF CONTENTS

2D Bi-dimensional
3D Three- dimensional

B

BCA Bicinchoninic acid
BSA Bovine serum albumin

C

CA Contact Angle
CO_2 Carbon Dioxide
$CHCl_3$ Chloroform
CF_3 Trifluoromethyl

D

DNA Deoxyribonucleic acid

E

EtO Ethylene Oxide

H

H_2O Water

L

LOC Lab-on-chip
LOP Lab-on-paper

M

M_w Molecular Weight

P

PBS Phosphate buffered saline
PDMS Polydimethylsiloxane
PE Polyethylene
PET Polyethylene Theraphthalate
PHA Poly(hydroxyalkanoate)
PHB Poly(hydroxybutyrate)
PLLA Poly L-lactide acid
PMMA Poly(methylmethacrylate)
PP Polypropylene
PS Polystyrene

S

SD Standard deviation
SEM Scanning Electron Microscopy

T

TiO_2 Titanium dioxide

U

UVO UV/ozone

X

XPS X-ray Photoelectron spectroscopy

W

WCA Apparent water contact angle

Z

ZnO Zinc oxide

LIST OF FIGURES

Chapter I.

Chapter II.

Chapter III.

Chapter IV.

Chapter V.

LIST OF TABLES

Chapter I.

CHAPTER I.

GENERAL INTRODUCTION

1 | Motivation and outline

Biomedical and biotechnological sciences have been continuously improving pharmacological products and biomedical devices. This progress has forced interdisciplinary work between the different classes of knowledges, including: biology, chemistry, materials science and engineering, physic, informatics and electronics [1].

The study of different materials and their properties has been very important to address the different requirements according to the final application. In this context, materials surface plays an important task in biology and medicine, as most biological reactions and interactions occur at interfaces [2]. Thus, surfaces properties such as chemistry, physical topography and exposed biochemical signals are partially responsible for the final performance of biomedical devices [3].

Superhydrophobicity has been recognized as a very interesting interfacial phenomena and, nowadays, is possible to find an increasing number of researching papers and commercialized products exploring the concept of high hydro-repellent surfaces [4-6]. The manifestation of this kind of surfaces came up with the observation of nature which has been asserting itself as a source of knowledge for the development of new artificial materials – biomimetism [7-9]. Over the last decades, the potential applications of these surfaces have grown in different directions, from automobile to biomedical industries [10]. Our interest goes essential to the biotechnological and biomedical applications, mainly for the conception of microfluidic devices and substrates for high-throughput analysis and for manipulation of liquid samples.

The framework of the present project consists on the surface modification of paper substrates to provide them superhydrophobic properties, inspired by the rough Lotus leaf surface. The purpose was to create different designs and shapes for open microfluidics, lab-on-chip (LOP) and devices for liquid's handling. For this reason, paper and the different potentialities that it offers appeared as a reasonable option. There are few studies reporting superhydrophobic (SH) surfaces using paper as initial substrate, far less than using materials as polystyrene (PS), poly(methyl methacrylate) (PMMA) and poly L-lactide acid (PLLA). We hypothesize that a bacterial origin polymer, the poly(hydroxybutyrate) (PHB), can be used to modify the topography of the paper surface, adding roughness to cellulose fibers surface at the micro and nano scales. Furthermore, as PHB is a low surface energy polymer, the modification may change the chemical character of paper surface. Different approaches will be addressed to modify the resulting substrates, with more

wettable domains. Additionally to bi-dimensional surfaces (2D), also three-dimensional (3D) substrates will be developed, in order to create structures for liquid manipulation.

This chapter reviews the literature concerning SH surfaces, focusing on the basic theory of wetting phenomenon, the main properties of these surfaces and their potential applications. Moreover, the literature reporting the usefulness of using paper as initial substrate for microfluidic purposes is also addressed.

2 | Bioinspiring systems: the concept of biomimetism

Materials science and engineering are important components for the development of biomedical devices, with a well-defined properties selection for each type of objects: density, mechanical properties, thermal conductivity, corrosion factors and wettability are some of the characteristics to take in account [1, 3].

One of the major current discussions in this field is the importance of taking advantage of the observations of nature and biological systems to reproduce interesting features on the design, adaptation or derivation of different synthetic materials, according to the target and the function-structure relationships [11, 12]. Otto Schmitt is the person who coined the word *Biomimetics*, when mimicked the electrical action of a nerve to create a new device (1957) [8]. The first official definition [8] appearing in a recognized dictionary describes biomimetism as *the study of the formation, structure or function of biologically produced substances and materials (as enzymes or silk) and biological mechanisms and processes (as protein synthesis or photosynthesis) especially for the purpose of synthesizing similar products by artificial mechanisms which mimic natural ones.* Despites of its new nomination, the looking of inspiration in nature remounts to 3000 centuries ago with the trials of the Chinese to make synthetic silk [9].

A considerable amount of literature has been published about biomimetic materials or processes, with different interesting characteristics such as superhydrophobicity and self cleaning, molecular-scale elements , energy conversion or conservation, high or reversible adhesion, drag reduction in fluid flow, self healing and sensory aid materials [7, 8, 13]. Biomimetic approaches have also been used in the development of materials and devices for health applications [14]. By way of illustration, Bhushan *et al.* [8] gave an interesting overview of different objects of nature and their characteristics with interest. Figure I.1 presents some biological systems that have been considered in biomimetic concepts.

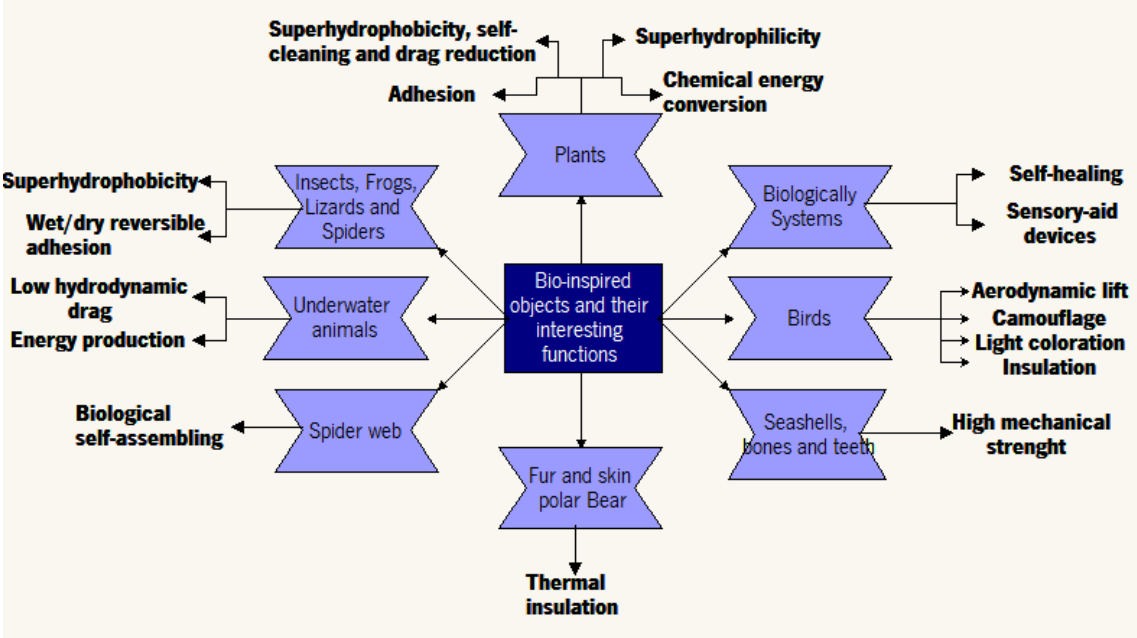

Figure I. 1 – Overview of different biological systems with interest in materials engineering, for biomedical purposes. Adapted from [8].

We focused on biomimetic materials whose interesting features are related with the topography of the surface. Some of classic examples are listed below, with a quick description:

- The skin of sharks (Figure I.2A) is composed by the narrow tooth-like structure of dermal denticles, parallel with the *in situ* flow direction, able to turn out and contribute to a faster movement in the water-hydrodynamic drag reduction. The understanding of this aquatic animal process enabled the creation of swimming suits to Olympic athletes, the increase of aircrafts speed and the production of polymer additives for drag reduction [5, 8, 15].
- The carnivorous plants (Figure I.2B) make use of their structure in slippery waxes layers to detain insects; indeed this phenomenon inspired the development of self-cleaning surfaces [8, 16, 17].
- There are a diversity of animals, specially insects and spiders with the ability to adhere on different surfaces promoting their locomotion. Based on it, new or derivable adhesive and reusable polymers can be used for tapes, toys, high technology and space industries [5, 8, 18].
- Other animals, like some amphibians (Figure I.2C) have the capability to move above wet domains without slipping, due to the nanopillar structure of each flat epidermal cell. Once again it can be used to design reversible and adhering materials, mainly in wet domains [8, 19].
- **Superhydrophobicity** observed in **Lotus leaf**, are discussed in detail below.

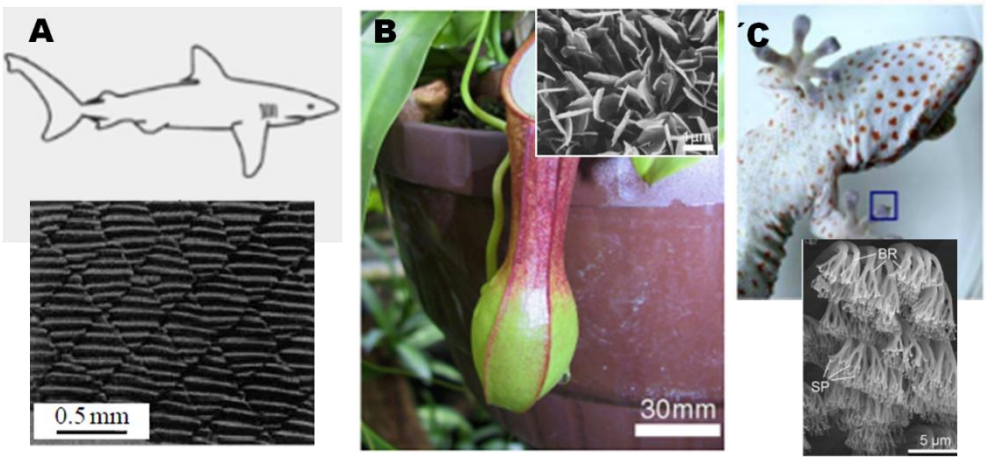

Figure I. 2 – Images of some inspiring biological systems, such as A) skin of shark at macroscopic scale and microscopic scales(adapted from [15]) , B) pincher plant with the respective SEM micrograph (adapted from [16]), C) Gecko's feet at macroscopic and microscopic scale (adapted from [19]).

The variability of topographies influences the differences observed in the properties of the interface of different plants and animals, concretely the wettability and particle adhesion. For instance, unlike lotus leaf, microscopic observations of *Magnolia grandiflora* leaf shown a smooth microstructure and is recognized as a wettable surface [8].

2.1. Natural superhydrophobic surfaces

2.1.1. Lotus leaf

In the beginning of the 90's, the observation of the water drops behavior onto Lotus leaf (*Nelumbo nucífera*) allowed the development of SH surfaces, which present properties as self-cleaning, drag reduction in fluid flow and low adhesion [5, 8, 9, 20-22]. The first studies were leaded by Barthlott and Neinhuis [20, 21]. Since eleventh century to today, the Lotus plant is admired by "*live in the silt but not sullied",* being a symbol of purity for Asian culture [9, 22, 23]. This means that these natural surfaces have the gift of let the water float on their leaves, carrying out the near pollution.

Superhydrophobicity is a physic term used to characterize lotus leaf-like surfaces with a high static contact angle (CA), greater than 150°, which exhibit extreme water repellence behavior and self cleaning properties [7, 10, 24]. The water drops roll of easily and remove any dirty debris present on the surface – the called Lotus leaf effect™ (note that this phenomenon was already patented as a trade mark in 1999) [10].

As reported by literature, the SH character of the Lotus leaf (see Figure I.3A*i*) may be associated with the hierarchically roughness structure at micro and nano scales and with the existence of a low surface energy wax coating. The systematic studies [20, 21, 25, 26] of the wetting properties of Lotus leaf revealed the presence of *papillose* epidermal cells randomly distributed onto the leaves surface, forming asperities with diameters from 5 to 10 μm and providing the microscale roughness. The nano roughness on the surface is found on the tridimensional epicuticular waxes that compose the apex of micropapillae and are usually denominated as branchlike structures (with diameters between 100- 200 nm) – see Figure I.3A*ii* [5, 8, 9, 27-31]. Therefore, the drop sits on the top of the nanostructure and the grooves are fulfilled by air bubbles providing high values of apparent CA [5, 24, 32, 33]. Besides the numerous studies that have attempted to explain the role of a hierarchical double- scale of roughness in lotus leaves, its effect is not yet completely clear [25, 30, 33, 34].

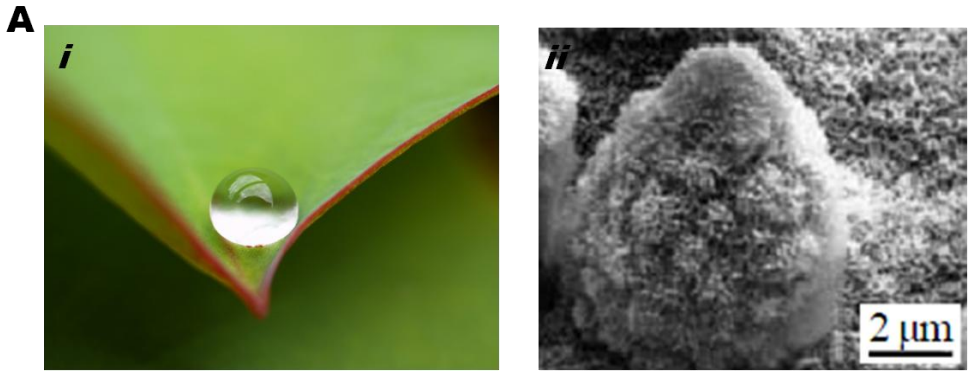

Figure I. 3 – A) Representative images of Lotus Leaf (*i*) and its respective SEM micrograph showing hierarchical organized topographies at micro and nano scales. Adapted from [8].

2.1.2. Other systems

Equivalent features are presented by other biological systems. The water striders (*Gerris remigis*) are recognized by their water resistant legs, being one of the only insects able to move quickly on water without getting wet. The topographical organization of the water striders legs is reported by some authors' studies: the observation of an oriented layer of tiny hairs (microstae) composed by nanogrooves and coated with cuticule wax was described. It confers water repellency properties to the water strider's leg. Also cicadas and butterflies are examples of non-wettable natural surfaces [7, 8].

3 | Artificial superhydrophobic Surface

During the last years, investigation groups all over the world studied hypotheses to fabricate biomimetic SH artificial materials. This has been promoted by the comprehension of the two leading factors: low surface energy and surface roughness [22, 28]. As the literature reveal, lower surface energy leads to less wettable surfaces. Nevertheless, water CA reached a maximum of 120° for smooth surfaces, an insufficient value to achieve superhydrophobicity, an a argument to conclude that a roughness surface is required to increase this value [35].

3.1. Fundamental Theories of the wettability phenomena

Thomas Young, a recognized polymath born at the ends of XVIII century, dedicated a huge part of his life for science research; one of his major studies was related the cohesion of fluids [36, 37]. Young's observations and experiments were crucial to recognize that for each combination of a solid and a liquid there is an appropriate CA formed between them, when exposed to the air [37].

One of the most important interfacial properties in materials science is the wettability of solid surfaces. The wetting phenomenon occurs when a liquid enters in contact with a solid surface and can be understood as a qualitative measure of how well the liquid spreads over the surface. It can be also determinated numerically by the measurement of the CA [11, 38, 39]. Flat, hemispherical and spherical are possible configurations of water drops onto the solid surface, depending on the surfaces tension between the different physical states. In Figure I.4 is possible to perceive the value of the contact angle θ resulting from the tangent angle between the solid – liquid and the liquid-vapour interfaces at the junction point of solid, liquid and vapour phases [11, 38, 39].

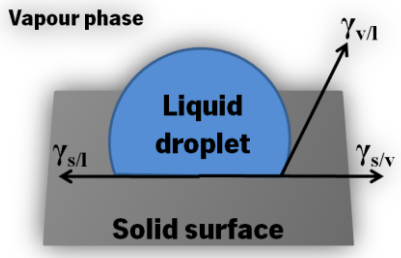

Figure I. 4 - Representation of the apparent contact angle. Adapted from [11].

When we consider a smooth, planar and chemically homogeneous surface, the acclaimed Young's equation can be simply applied to correlate the contact angle θ and the involved interfacial energies, as described by Equation I.1 [38, 39].

$$\cos\theta = \frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}} \qquad (I.1)$$

Note that the surface tension γ_{SV} (solid/vapour) is associated with the spreading of the drop over the surface, whereas the surface tensions γ_{SL} (solid/liquid) and γ_{LV} (liquid/vapour) are related with the contraction of the drop [11]. It is possible to determinate the experimental value of CA, usually using a goinometer, and the surface free energy of the liquid by different techniques, such as the well - known ring method [38].

If the CA is below 90° the surface is calling hydrophilic; otherwise it is hydrophobic [40]. There are still place for the extreme cases: when the CA is equal or less than 10° means that we are in the presence of a superhydrophilic surface and when CA is equal or more than 150° we achieved a superhydrophobic state [40], The last one, as already referred, is the bottom line of our work.

The control of wetting phenomenon is an eminent concern in surface engineering [39], where surface free energy and surface topography are the primary parameters that influences wettability – see Figure I.5 [11, 32]. Thought less significant, the cleanliness of the surface also affects how wettable it is [32, 41]. We will focus on the roughness and low free energy surfaces as main keys to produce SH artificial materials.

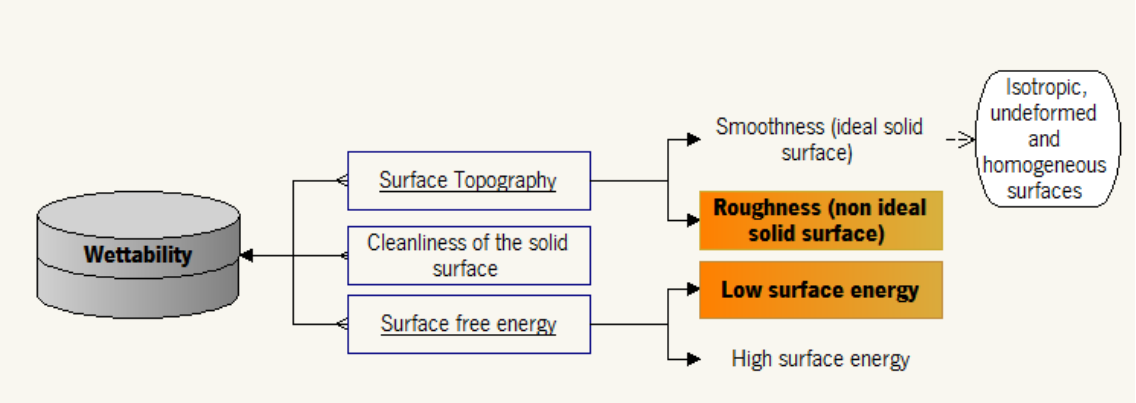

Figure I. 5 - Diagram with the main factors influencing the wettability.

It is well-established that real perfect smooth and uniform surfaces are not always conceivable and when in presence of topographical roughness the Young's equation is not valid anymore [39]. At this position it is necessary to make use of other models such as the ones suggested by Wenzel and Baxter and Cassie [9, 11, 42]. Herein, the correct designation for the angle measured should be defined the apparent contact angle [11, 43]. A short review of the referred models is given below.

3.1.1. Wenzel's Model

In 1936, Wenzel [44] assumed that the effect of the roughness on the solid must be taken into account. He argues that when a liquid drop enters in contact with a non planar surface it could totally fill the grooves (see Figure I.6A) [9, 11, 43, 44]. Introducing the roughness factor *(r),* the system will suffer a variation on the free energy: based on Young´s equation, Wenzel proposed a new equation [9, 11, 43, 44]:

$$\cos\theta^* = r\cos\theta \qquad \text{(I.2)}$$

Where θ^* corresponds to the CA of the rough surface under the Wenzel's model (apparent contact angle) and r is the ratio between the actual area of the solid/liquid interface with the smooth projected area (disregarding the roughness), being always equal or higher than 1. Considering the CA of the projected smooth surface (θ) higher than 90°, θ* will be larger; otherwise when the θ is lower than 90°, θ* will be smaller [9, 11, 44].

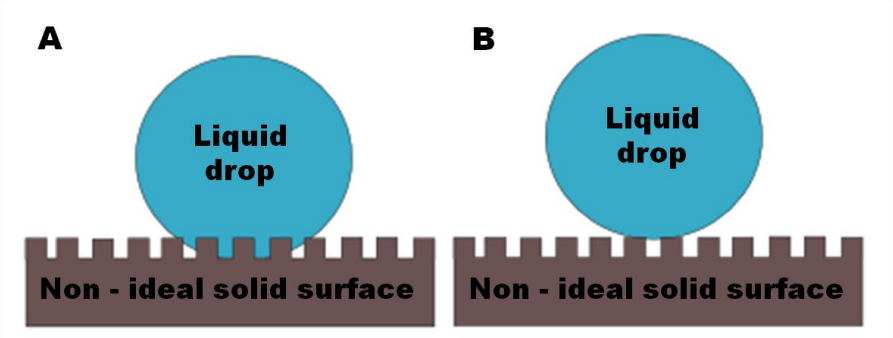

Figure I. 6 - Schematic of A) Wenzel's model and B) Cassie and Baxter's model. Adapted from [9].

3.1.2. Cassie – Baxter's Model

Extending the Wenzel's studies, in 1944, Cassie and Baxter [45] reported a different model for heterogeneous wetting (surfaces formed by different class of chemical substances or porous surfaces). In their studies, they assumed that the rough solid surface is composed by solid and vapour parts, both well-distributed in pint-sized fractions (see Figure I.6B). It must be noted that the vapour part of the solid surface corresponds to the air pockets trapped between the rough details of the surface [9, 11, 45].

Accordingly, Cassie and Baxter considered two area fractions: one corresponding to the solid state of the surface (f_1) and other to the vapour part of the solid (usually referred as air pockets) (f_2); additionally, for each one, they defined the apparent contact angle (θ_1 and θ_2, respectively) [9, 32, 45]. A new equation was formulated:

$$\cos\theta^* = f_1 \cos\theta_1 + f_2 cos\theta_2 \quad \text{(I.3)}$$

Since the CA between air and water (θ_2) is 180° and the different parts of the solid surface are well-distributed ($f_1+f_2=1$), is possible to simplify the equation [9, 32, 45]:

$$cos\theta^* = f_1(cos\theta + 1) - 1 \quad \text{(I.4)}$$

Contrasting with the Wenzel model, whatever the CA on a smooth surface, the apparent contact angle can still be increased [9, 32]. Besides the wide range of these model's applications, different researcher teams investigated the limitations associated [46-48]. They argue that there is no one single value of CA for a rough/heterogeneous surface, but a range of values, between the receding and the advancing contact angle [32, 46, 48]. A difference between the advancing (θ_{adv}) and the receding (θ_{rec}) contact angles (CA hysteresis) happens when the contact angle line can assume many equilibrium shapes, governed by the competing forces [39]. It represents a measure of energy dissipation and the related literature describes a low CA hysteresis for non-adhesive water repellent surfaces [32].

We decided not to detail these arguments, and adopt the Cassie and Baxter's Model to explain the values obtained in this work. As Gao and McCarthy [46] pointed out: the Wenzel and

Cassie equations *"should be used with the knowledge of their fault".* The principal argument for adopting such model is that the original smooth material used presents typically CA < 90°. .

3.2. Surface Free Energy

The surface free energy affects significantly the wettability and is determinated by the chemical composition of the solid surface [11, 49, 50]. When the interface solid-liquid-vapour is formed, the equilibrium of the intermolecular bounds is disrupted due to an excess of energy that minimize the surface area - surface free energy [38, 51].

The larger the surface energy is, the easier the surface is wettable. Normally the highest energy surfaces corresponding to ordinary metals and their products (oxides, sulfides and inorganic salts) and can be wetted by regular liquids; however organic solids and polymers (usually bounded by van der Walls forces or hydrogen bounds) represent the surfaces with lowest energy [11, 39].

The relationship between the variation of the chemical composition and consequently the system's surface free energy and the wettability of the surface has been widely investigated since Zisman first conclusions [52]. The strategy to increase the γ_c can include the modification of the chemical composition of the polymer surface, by specific treatments skilled to add or replace some functional groups [11]. For example, contrarily to the introduce of nitrogen or oxygen atoms, the adding of fluorine atoms into the hydrocarbon chain decreases the γ_c of the polymer [11].

3.3. Self cleaning effect and other interesting properties

The self cleaning property is already associated with SH surfaces. It encompasses the no contamination and the dirty-free ability of the surfaces, desirable for a range of applications, from military, to industry, technology, biomedical, agriculture and every day uses (antifogging, anti-icing, anti-reflection, corrosion resistance, drag reduction, exterior construction materials, sensor, solar cell, textiles, and other fields are described) [53, 54].

Today, a route to self-cleaning are being intensely studied : remove the dirty by the rolling of droplets [53]. Nature provides some examples of no wettable self cleaning surfaces where the droplets of water running off the substrate (non-adhesive superhydrophobicity) [54].

According with the mechanism of self-cleaning, there are different approaches to produce self cleaning surfaces. Different studies [54-56] reported the fabrication of TiO_2- based self cleaning surfaces with origin on photocatalysis and photo-induced superhydrophilicity. The *gecko* foot

inspired some authors to develop dry-self cleaning substrates [54, 57, 58]; some underwater organism motivated researchers to create substrates with anti-fouling properties [54, 59, 60]. Although, as already mentioned, *lotus* self-cleaning effect was our inspiration: contaminated particles can be easily removed by the water droplets rolling kinetics.

Besides self-cleaning ability, depending on the application, there are other desirable properties for these substrates, such as flexibility, breathability, reversibility and anisotropy [28]. Chemical resistance can be also an important requirement. Guo *et al.* [61] suggested the possibility to obtain anti-corrosive characteristics from certain materials (cooper, steel or alloy aluminum), showing SH behavior in a wide pH range. Despite the increasing of roughness is not favorable for good optical transparency, this one is still very desirable for car widescreen and eyeglasses: the efforts to arise a middle ground, where the two properties can co-exist, are commonly found in literature [62-65]. For electrical devices, such as some of microfluidic, the electrical conductivity is clearly an interesting feature. As metal and metallic nanomaterials are naturally charged, it is easy to understand their application to obtain conductive SH substrates [66, 67].

The intrinsic relation between water-repellency and self-cleaning can be explained by the topographical asperities of the surface. Barthlott and Neinhuis [20] suggested that onto a smooth substrate the dirtiness is predominantly redistributed by a water drop or flow; otherwise onto a rough substrate it get trapped to a water droplet and is automatically removed off the surface (see Figure I.7A and I.7B, respectively) .

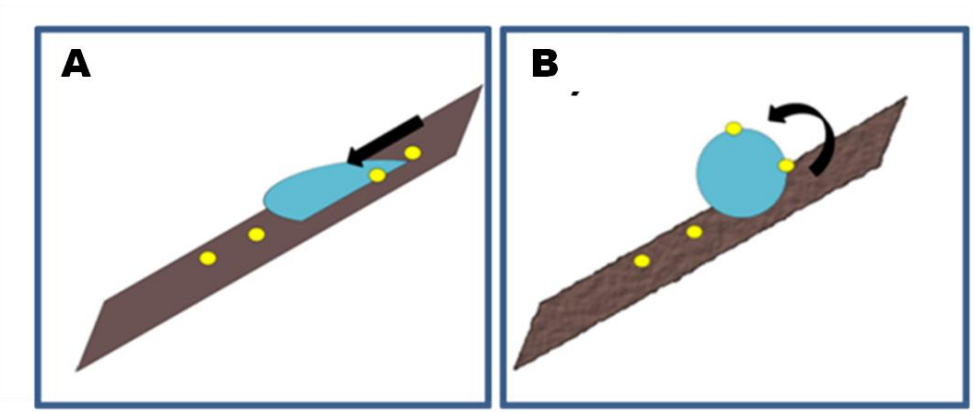

Figure I. 7 - Comparison of liquid droplet onto A) smooth and B) rough surfaces. Adapted from [11].

3.4. Strategies and employed materials to produce superhydrophobic surfaces

3.4.1. General considerations

To produce artificial SH surfaces, many materials and fabrication strategies have been employed [10, 28, 68]. Until recently, the most reported processes required expensive equipments and complex methodologies [10].

The SH fabrication are generally categorized into two approaches: creating a topography at micro and nano scales on a low surface energy substrate or chemical surface modification of rough substrates with low surface energy agents [10, 26, 28, 69]. For the first approach, most used materials are restricted to fluorocarbons, silicones, some organic (such polycarbonate, polystyrene, alkyl ketene dimer) and inorganic substrates (such zinc oxide (ZnO)). These substrates are generally processed with one-step techniques [10, 28] . According to the properties of the substrate, both approaches can be simultaneously suitable to render a SH surface, amplifying the range of applications. Some of the typical methods and materials reported by literature to fabricate SH surfaces are described in Table I.1.

Table I. 1 - Some methodologies and employed materials to produce SH surfaces

Superhydrophobic fabrication technique	Most cited initial substrates	References
Lithography	Silicon wafers, gold	[70-73]
Plasma treatment	Teflon, polyethylene theraphthalate (PET), Cellulose surfaces, polydimethylsiloxane (PDMS)	[74-78]
Chemical Etching	Aluminum, copper, zinc	[79, 80]
Electrical/chemical deposition	Cotton textiles, cellulose Fibers, cooper	[81-83]
Electrospinning	PS, copolymer PS-PDMS	[84-87]
Layer-by-layer	Polyimide	[88]
Crystallization control	Isotactic polypropylene (PP), polyethylene (PE)	[89, 90]
Electrohydrodynamics	PS	[91]
Sol-gel process	ZnO, Alumina gel- films, TiO_2	[92-94]
Phase separation	PS, PLLA	[95-100]

It is possible to decrease the surface free energy just by modifying the chemical composition: different methods have been selected, namely involving the introduction of specific surface reactive molecules such alkyl thiols or fluorinated organic silanes [3, 28, 101]. Using a fluoroalkylsilane coating (FAS), with a CF_3 –terminated molecule, Teshina *et al.* [75] grafted organosilane molecules on the hydrophilic groups previous introduced in poly(ethylene terephalate) (PET) substrate.

3.4.2. Phase-separation methodology

The methodology employed in this thesis is based on a phase separation approach. When compared with other methodologies, the phase separation congregates some advantages: it requires less expensive apparatus and materials, is possible to have a easier control over every part of the process, to obtain an acceptable homogeneity of the treatment and to accomplish the surface modification in large areas, even on complex substrates [10, 102, 103]. The phase separation process can be triggered by a range of different forces. We focus on the non solvent induced phase separation (or wet phase separation). The basis is that the immersion of a homogeneous polymeric solution into a third component (non solvent), which is miscible with solvent, creates a ternary system.

The immersion of the polymer solution into a coagulation bath generates the exchange of solvent and non-solvent, at the polymer solution – non solvent interface. This causes instability on the thermodynamical state of the system [104-106]. In order to attain the minimum Gibb's free energy, the system replies with the separation in two coexisting phases: polymer poor phase and polymer rich phase [105, 107, 108]. The polymer poor phase is characterized by the precipitation of polymer nuclei; thus, the rich phase originates the polymer matrix aggregated around the nuclei structure [105, 108]. There are some studies reporting the use of a phase separation methodology to fabricate SH surfaces: Li *et al.* [109] used a solvent-non solvent methodology to create a lotus-like structure onto a poly(vinyl chloride) substrate; Song *et al.* [110] used a similar approach to obtain PLLA SH surfaces. Therefore, this versatile method is capable to provide a micro and nano texture to different materials. Although, it is important to consider that different parameters can influence the morphology of the formed structure such as polymer concentration, system's temperature, the solvent and non-solvent selection and the addition of particular additives [105, 111, 112].

3.4.3. Paper

Paper detains a place of excellence in human history. There are a wide range of possible applications in our daily lives and new routes of using paper have been conceived day-to-day [113-115]. This ubiquitous material is composed by cellulose fibers arranging in a bi-dimensional random network, which presents a hydrophilic and hygroscopic behavior responsible by the readily adsorption of water and moisture; with the increase or decrease of moist air the paper swells or shrink, respectively [115, 116]. Different properties make paper an interesting material for biomedical applications such biodegradability, low-cost, flexibility, renewability, disposability, the wide availability and the easiness to be functionalized with other chemical compounds [113, 117-120]. The possibility to produce SH paper has a great potential to enhance the possibilities of using this material.

4 | Biomedical uses of biomimetic superhydrophobic materials

Researching on bio-inspired SH surfaces has received a surge of interest in the last two decades. Currently, there are already some products commercialized and even more published patents regarding water repellent surfaces [24, 121]. For example, Schoeller Textil AG developed a new technology based on nano and micro structures: nanoparticles of silica (or other polymers) that can be incorporated on clothing fibers, providing no dirty clothes - NanoSphere technology® [122].

Figure I.8 summarizes the main biomedical related applications associated to biomimetic SH surfaces. The possibilities explored in this thesis are related with microfluidic devices and lab-on-chip (LOC), which has received much attention in last few decades [123].

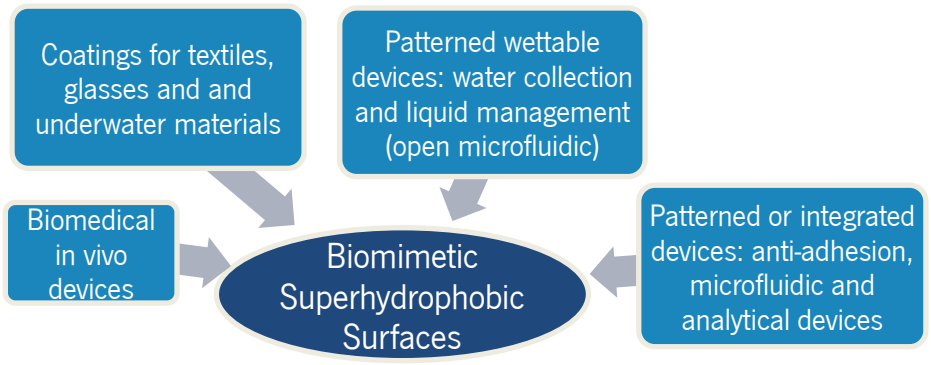

Figure I. 8 – Possible applications of biomimetic SH surfaces.

4.1. Microfluidic devices

Microfluidic platforms are able to sample, monitor, transport, mix, react and analyze different fluids [123]. Diverse designs have been reported, according with the target use. Among other, the advantages of using microfluidic devices are shown in Figure I.9 [123, 124].

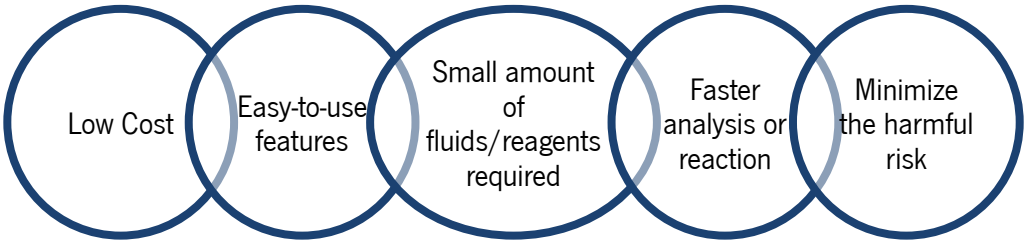

Figure I. 9 - Main advantages of using microfluidic devices.

Traditional microfluidics devices make use of materials like glass, metals, quartz and silicon. Also the use of polymers such as PMMA, polydimethylsiloxane (PDMS), polyethylene (PE) or PS has been suggested as a promising alternative to traditionally employed materials, thanks to the relative low-cost and biochemical compatibility [123, 125]. Meanwhile, many advances have been made on micro and nanofabrication: Whitesides and his co-workers [126] used PDMS to develop a microfluidic device, using a soft lithography technique to control the topographical structure of the surface. Nowadays, the most part of commercialized microfluidic devices present closed microchannels (with a roof). To permit an easier and direct access to the channels, to prevent the bubble trapping and obstruction and to facilitate the fabrication process, a new generation of microfluidics has been emerging: open-air microfluidic devices [127, 128]. Different approaches have been suggested as confining forces to the liquid flow, such as mechanical pumping, gravity or the use of magnetic fields [128].

SH substrates may be potentially used in open microfluidic and LOC devices if geometrical features with distinct wettabilities are patterned onto these surfaces to circumscribe the position or the flow of the liquids. For instance Oliveira *et al.* [98] suggested the fabrication of open microfluidic devices through the surface modification of patterned SH polystyrene using UV/ozone (UVO) irradiation to increase the wettability of channels-like domains. In this work we intend to expand such concept using SH paper and explore other surface modification methodologies.

4.2. Lab-on-paper (LOP)

The bottom line of our researching work is the use of paper as the substrate. Pioneer Whitesides team's researching work [129] (2007) suggested the use of a photolithography approach to create a patterned paper for portable bioassays purposes. This revolutionary idea was the beginning of a new researching field – LOP. Since then, innumerous approaches have been proposed, from two to three dimensional designs (see Table I.2).

Table I. 2 - Different approaches to create patterned paper surfaces

Applications	2D structures		3D structures	
Bioassays and chemical analysis	Lithography	[129-133]	Assembling of layers of patterned paper and tape	[118, 134]
	Cutting special features with computer-controlled knife	[135]	Creation of "switches" by cutting flaps over flow channels	[136]
	Plasma treatment	[136, 137]	Origami-based patterned paper	[138, 139]
	Inkjet printing with specific agents	[140-143]	3D paper-based electrochemiluminescence immunodevice	[144]
	Wax printing	[145-151]		
	Flexographic printing	[152, 153]		
	Computer controlled- CO_2 laser to selective hydrophobization	[154]		
Traditional laboratory apparatus	Patterning of micro-areas on paper – microzones plate	[154-157]		
Handling of fluidic samples	SH paper patterned by a inkjet printing approach	[76]		

All the studies reported above are based on hydrophobic/hydrophilic contrast: more wettable domains are encased in less wettable limits, forcing the liquid to flow and to be fixed in pre-determined positions. Among all methodologies, printing techniques have been the most described, differing in some parameters such as the hydrophobic agent and the printing type [141,

145, 152, 158]. With the development 2D substrates appeared the first reports of 3D paper devices. Some researchers followed this new route [139, 159, 160]: Govindarajan *et al.* developed a 3D origami device to extract bacterial DNA with the possibility to access any layer at any time, just by unfolding the device [138]; Ge *et al.* [144] designed a wax-patterned 3D paper based electrochemiluminescence device to the diagnosis of tumor markers. Additionally, new ways of using it are now starting to be employed in the reproduction of conventional laboratory apparatus, such as microplates targeted to perform particular assays [155-157].

4.3. Superhydrophobic Paper Substrates

Until now, few researches have addressed the use of SH paper to create LOP systems: Balu et al. [77] modified the paper surface through selective etching by oxygen plasma followed by the deposition of a thin film from pentafluoroethane; Barona et al. [142] sprayed a paper surface with a nanocomposite film. Both used an inkjet printing methodology to pattern the paper substrates, for LOP and fluidic handling purposes**.** We hypothesize that SH paper could be used to produce patterned surfaces with higher regions featuring contrast in wettability and could be used in the production of 3D devices with self-cleaning and water resistance characteristics.

5 | References

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CHAPTER II.

MATERIALS AND METHODS

1 | Materials

In elapsing of this thesis work, we used two types of paper, printing paper with a density of 80 g/m^2 and cartridge paper with a density of 125 g/m^2. The A4 paper sheets were kindly provided from Staples® and Pontus®, respectively. The paper substrates were cut in squares of 4, 9 or 16 cm^2 or with 2D projected-shape of the 3D structures, according to the purpose. Among the main characteristics of the used paper are the white color and a thickness ranging from 0.17 to 0.18 mm. The molecular structure is shown in Figure II.1.

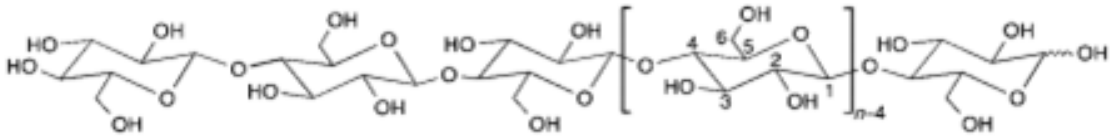

Figure II. 1 – Molecular structure of cellulose [1].

The aliphatic polyester poly(hydroxybutyrate) (PHB) has bacterial origin and was supplied by Biomer Inc (Germany) in a white powder form. This is a high molecular weight (Mw=230 kg/mol) thermoplastic, which belongs to poly(hydroxyalkanoates) family (PHAs) and has a huge potential as renewable and biodegradable polymer [2-5]. The degree of crystallinity is about 60% and the melting point is around 175°C. Other interesting properties of PHB, which permit the application in different fields, are the isotactic configuration, the non inclusion of any catalyses residues, the optical activity, the no solubility in water and the lower permeation for oxygen, carbon dioxide and water (hydrophobic polymer) [5-8]. Additionally, when exposed to moisture the rate of degradation is insignificant making PHB appropriate for different uses [7]. The molecular structure of PHB is represented on Figure II.2.

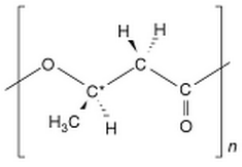

Figure II. 2 – Molecular structure of PHB [9].

This polymer has been investigated to be applied in different fields as food package and biomedical applications (tissue engineering, scaffolds, sutures and drug delivery) [6, 10-13]. As referred above, the PHB is not soluble in water so other organic solvent must to be used. The

chosen solvent was chloroform ($CHCl_3$) and it was obtained from Sigma-Aldrich (p.a≥ 99%). The absolute ethanol (p.a≥99.5%) was supplied by Pancreac and used as non solvent.

2 | Methods

2.1. Production of superhydrophobic paper surfaces

The first aim of this project was the production of SH paper substrates. Therefore we decided that the best procedure to readily and simply obtain SH paper was a phase separation based on non solvent action. The processing is illustrated in Figure II. 3.

As previous mentioned the paper substrates were arranged cutting squares of 4, 9 and 16 cm^2 and the 2D projections of the different structures and immersed these ones in chloroform, at least for 6 hour, to remove any possible additives soluble in this organic solvent. Meanwhile, a 7.5% (w/v) PHB in chloroform solution was prepared and gently heated to guarantee the total polymer dissolution. Then the paper specimens were immersed on the PHB solution during 6 hours. Subsequently and for a minimum period of 12 hours the paper samples were promptly transferred to a coagulation bath composed by non-solvents of PHB: absolute ethanol and distillated water (85:15 v/v). Afterwards the paper surfaces were left to dry at room temperature and atmospheric pressure, obtaining rough paper substrates.

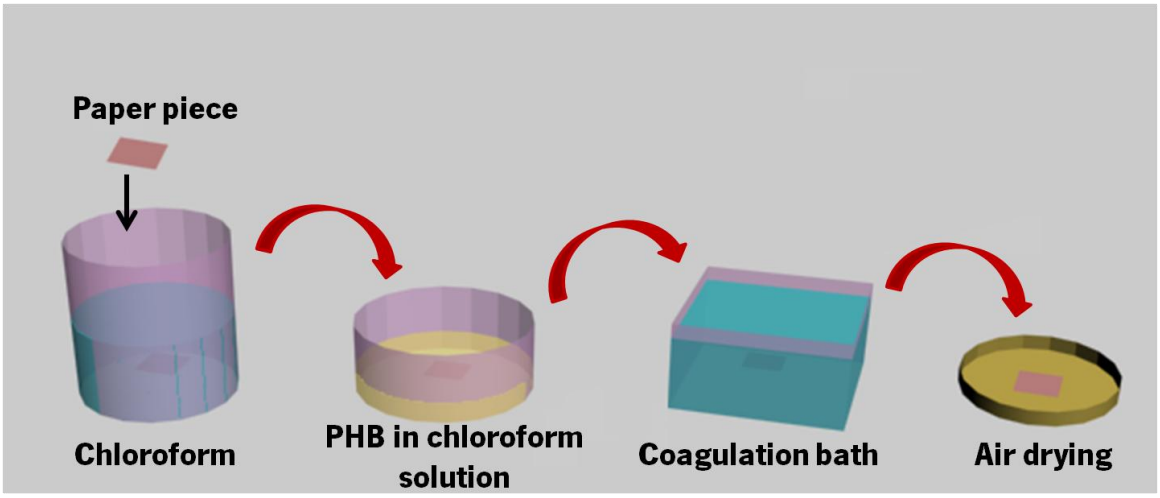

Figure II. 3 – Representation of the different steps to produce a SH paper.

2.2. Surface Characterization

2.2.1. Scanning Electron Microscopy (SEM) analysis

SEM analysis has emerged as a powerful tool to provide topographical images of samples surface with high resolution. This system is based on electron – surface interactions that generate signals able to give detailed visual information about the surface morphology at micro and nano scales and the crystalline structures on surface [14, 15].

Therefore, the observation of the surface roughness was performed by a Leica Cambridge S-360 scanning electron microscope, from Leica Cambridge (UK), at an accelerating voltage of 3 and 5 kV and different magnifications. All samples were precoated with a layer of sputtered gold to induce the required conductivity to electron – surface interactions.

2.2.2. Apparent water contact angle (WCA) measurement

The wettability of the surfaces was evaluated by the apparent water contact angle measurement, using an OCA15+ goniometer apparatus from DataPhysics (Germany). The measurements were carried out at room temperature, with ultra pure water (HPLC grade). Following a sessile drop method, a motor–driven syringe dispensed a 5 µl drop in the sample surface and the fitting of the shape was performed by SCA20 software. At least three measurements of each condition were carried out on surface material, usually one day after the treatment. The pictures were taken 10 seconds after the initial contact drop-surface.

2.2.3. X-ray Photoelectron Spectroscopy (XPS) analysis

Other important technique widely employed to characterize a sample surface is XPS. This analysis is governed by the biding energy and intensity of the photoelectron peaks, emitted from the surface when exposed to a mono-chromatic x-ray [16, 17]. The XPS information provides the chemical nature and quantification of certain element, within a probing depth about 10 nm [16, 18].

To conduct the XPS analysis we used a VG-Microtech Multilab 3000 spectrometer from VG scientific, equipped with a hemispherical electron analyzer and an x-ray tube with Mg and Al anodes.

2.2.4. Mechanical behavior – robustness test

Mechanical wear is a special concern for SH substrates. The handle contact may cause damages on the topographical morphology of the surface and also the surface contamination, decreasing the lifetime of the substrate. Therefore, it was important to evaluate the robustness of the prepared SH substrates; we decided to carry out a simple non-standard mechanical test with the finger [19], forcing it down the surface.

2.2.5. Sterilization procedure

To entirely fulfill the requisites of general laboratorial material, the SH paper surfaces must be resistant to at least one sterilization technique. Squares of common, SH and patterned paper with 1 cm^2 of surface area were sterilized by ethylene oxide (EtO) at Pronefro (Portugal). Standard conditions involved a working temperature of 45°C, a cycle time of 14 hours, a chamber pressure of 50 kPa and a humidity level of 50%.

2.2.6. Protein Adsorption study – BSA assay

The understanding of the principles of protein absorption onto the material surfaces is fundamental to project a medical product or device. In an effort to reinforce the potentiality of the present thesis project we carried out a protein absorption study, with Bovine serum albumin (BSA from Sigma- Aldrich) protein to compare the SH paper and the common paper (cartridge type).

This was done by means of a micro bicinchoninic acid (BCA) protein assay kit (Pierce Chemical Co, USA) for the quantification of total protein. According with the literature [20] and the provider instructions, this assay is based on the reduction of Cu^{2+} to Cu^{1+}, when in the presence of a protein in an alkaline environment. The chelation of two molecules of BCA with Cu^{1+} forms a kind of purple product reaction that exhibits strong absorbance at 562 nm. The absorbance must be linear with increasing protein concentration.

At the beginning, all the samples were cuted in circles with a diameter of 10 mm. The specimens were fixed on the bottom of 12-well plates and immersed in 3 ml of different BSA concentrations in phosphate buffered saline (PBS from Gibco), following the scheme illustrate in Figure II.4. Three samples of both rough and smooth paper were used for each concentration. The BSA absorption rate was evaluated for the first hour and we used the same initial concentrations to study both types of paper surfaces.

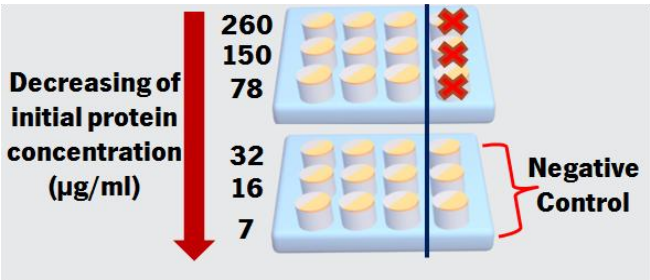

Figure II. 4– Representation of protein adsorption's study, with the initial concentrations used for both type of substrates (SH and common paper).

Meanwhile we prepared the standard of BSA with known concentrations. After an incubation period of 2 hours in a working reagent at 37˚C and 5% of CO_2, the absorbance was read at 562 nm on a Microplate Spectrophotometer (Tecan). The construction of a calibration curve for BSA provided a linear relation that allows the calculation of the protein concentrations corresponding to the read absorbance.

2.3. Surface Modification

In order to pattern the SH paper surface, a chemical modification was required. Thus, we used plasma and UVO irradiation treatments to modify the wettability in certain regions of the surface.

In the first part of the work the surface modification was performed using a Plasma Prep5 chamber from Gala Instruments (Germany), with the pressure kept between 0.16 and 0.2 mbar. All the samples were exposed at an argon working atmosphere with an applied voltage of 2.2 V. The exposure time varied between 5 and 180 seconds. Wettable channels were patterned in the initial SH paper. The samples, were covered with two glass slides, spaced 1 millimeter between them, and treated with argon plasma irradiation. The exposed region of the substrate (not covered by the glass) is expected to acquire more wettable characteristics.

An alternative solution to plasma treatment was achieved using a UVO ProCleaner from Bioforce Nanoscience Inc (USA), for exposure times ranging from 1 to 30 minutes. Distinct geometries were patterned on the samples surface, emphasizing the multifaceted ability to create low-cost designs. For the UVO treatment we used acetate polymeric sheets that were cut with desirable hollowed geometrical features. By putting such masks over the SH paper, the cut-out part of the acetate sheet exposes confined regions of the sample to the UVO light, increasing the wettability in these regions.

After the treatments the samples were stored in a dry environment until characterization.

Also writing, printing and stick-on flat objects were here considered as innovative tools to modify and pattern the SH paper surface. We investigated simple water-based markers (Uso®), coal pencils and colored pencils (USO®), stick-on circles (Uso®) and printing models to pattern the substrates with more wettable domains. The printing models were developed in Microsoft Word 2007® software and printed in a standard and commercially available Inkjet Printer (Epson Stylus SX105).

We used standard areas (4, 9 and 16 cm^2) to prepare SH paper structures. The photos were taken by a Panasonic photographic digital camera (Lumix FS14). Different models were used.

2.4. 3D superhydrophobic paper architectures

Different structures were engineered using the developed SH paper, in order to reproduce some conventional labware, that can be obtained by basic origami or cut/glue methods.

2.5. Statistical analysis

All data are described as a mean ± standard deviation. Statistical significance was analyzed using the one-way ANOVA test, with $p<0.05$ considering differences as statistically significant.

3 | References

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CHAPTER III.

MODIFICATION OF PAPER USING POLY(HYDROXYBUTYRATE) TO OBTAIN BIOMIMETIC SUPERHYDROPHOBIC SUBSTRATES

MODIFICATION OF PAPER USING POLYHYDROXYBUTYRATE TO OBTAIN BIOMIMETIC SUPERHYDROPHOBIC SUBSTRATES

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Abstract

Inspired in nature, the creation of synthetic superhydrophobic surfaces is nowadays a major object of study, with many potential applications in different fields. The fabrication of such substrates has been dominated by the use of non biodegradable and poorly flexible materials, using expensive and complex procedures. To overcome this issue, we propose a simple concept for fabricating low-cost, biodegradable, and flexible biomimetic superhydrophobic materials, using paper as substrate. The methodology includes the precipitation of poly(hydroxybutyrate) (PHB) on the surface of cellulose fibers of papers using a phase separation process. The obtained surfaces showed a rough texture, at both micro and nano length scales and an apparent water contact angle of 153.0±0.7˚. Furthermore we showed that argon plasma treatment increases the surface wettability and it is possible to control the wettability in certain regions by using adequate hollowed masks. Such findings could be used for the production of cheap open-microfluidic or lab-on-chip devices, using materials from renewable resources.

Keywords: Superhydrophobic surfaces; biomimetic; patterned paper; microfluidic devices

1 | Introduction

The development of new devices for microfluidics, electronics, biosensors, environmental and biomedical applications requires often substrates exhibiting low adhesion and non-wetting properties [1-4]. Many surfaces found in nature exhibit highly hydrophobic and self-cleaning properties, including the leaves of plants such as lotus, the wings of cicada and butterflies or the water strider´s leg, which have inspired many researchers in understanding the underlying phenomena and develop synthetic surfaces with similar properties [5-8].

It has been recognized that superhydrophobic surfaces (apparent water contact angle, WCA, higher than 150°) must combine two essential properties: (i) surface roughness, especially hierarchically roughness at the nano and micro scale-levels and (ii) low surface energy [1]. Synthetic superhydrophobic substrates have been produced by a variety of methodologies, from chemical modifications treatments that lower the surface energy (normally adding fluorides compounds to the surface) [9] to the introduction of a double-scale of roughness features, mimicking the lotus leaves topography [4, 7, 10-12]. Superhydrophobic surfaces have been produced using metallic, inorganic and polymeric substances. Many applications do not require long-term use of such kind of substrate, such as package materials, platforms for diagnosis and biosensing or a serie of biodegradable devices for biomedical and environmental applications [13-15]. Biodegradable or disposable superhydrophobic substrates produced by natural or synthetic polymers have not been extensively developed. For example, substrates from poly(L-lactic acid) [16, 17] or chitosan derivatives [18] fabricated using a phase separation method, were reported in literature. The important role of paper in the scientific and technological world has been recognized mainly to its effective potential as a cheap, biodegradable, versatile and flexible substrate, to be used, for example, in microfluidic analytical devices or in a series of other lab-on-paper applications [13, 19, 20]. Concretely, through the pioneered work of Whitesides and co-workers, 2D or 3D structures based on a hydrophobic-hydrophilic patterned paper were suggested for low cost bioassays devices [21, 22]. We hypothesize that such lab-on-a-chip devices, as well as substrates for packing, sensors or paper-based MEMS, could be highly improved if one could start with surfaces exhibiting extreme wettability. A few attempts reported the preparation of paper showing superhydrophobic characteristics: Yang and Deng coated paper with silica nanoparticles that were then chemically modified with 1H, 1H, 2H, 2H – perfluorooctyltriethoxysilane [23]; Balu *et al.* modified the paper surface through selective etching

via oxygen plasma followed by the deposition of a thin film from pentafluoroethane [24]. Such methods permitted to obtain substrates exhibiting WCAs higher than 150°. In this work we present a simpler method of modifying paper by depositing a thin film of a biodegradable polymer, poly(hydroxybutyrate) (PHB), with an adequate micro/nanostructure to provide superhydrophobic characteristics to the obtained substrate. The generation of such particular topography in PHB is achieved through a phase separation methodology, which was applied before in poly(L-lactic acid) [17] and polystyrene [25].

Our inexpensive and simple bench-top method can be easily accessible to virtually any researcher and may be scaled-up to higher massive productions. Moreover we show that further plasma treatment on such substrates permits to decrease the WCA down to superhydrophilic regime in a controlled way, just by adjusting the exposure time. The plasma modification may be spatially controlled in order to confine such modification in certain regions of the substrate, enabling patterning hydrophilic lines surrounded by superhydrophobic regions that could be used to generate gradients in fluid stripes [26] or in devices for open-microfluidic applications [2, 25, 27].

2|Materials and Methods

2.1. Materials

Commercial 80g/m^2 copy paper, purchased in Staples® Office Center, was used as supporting substrate. Commercial grade poly(hydroxybutyrate), PHB, with a melting point of 173 °C, was kindly supplied by Biomer in the form of a very thin powder. Chloroform and ethanol, with 99% and 99.5% of purity, were supplied by Carlo Erba Reagent and Pancreac, respectively.

2.2. Methods

2.2.1. Production of superhydrophobic paper

Squares of 4 cm^2 were cut from A4 sheets of commercial copy paper and were immersed in chloroform for at least 6 hours, to extract any possible additives soluble in the solvent. Then the samples were immersed for 6 hours in a 7.5% (w/v) solution of PHB in chloroform, followed by an immersion in a coagulation bath, formed by a mixture of 85/15 (v/v)

of ethanol and water, during 12 hours. Finally the samples were placed in a glass surface and dried at room temperature.

2.2.2. Surface Modification

A Plasma Prep5 chamber (Gala Instruments, Germany), was used to modify the surfaces wettability. The pressure was kept between 0.16 and 0.2 mbar. All the samples were exposed in an argon working atmosphere with an applied voltage of 2.2 V. The exposure time varied between 5 and 180 seconds. After the treatment the samples were stored in a dry environment until characterization.

Wettable channels were patterned in the initial superhydrophobic paper – see scheme in Figure 1. The samples, were covered with two glass slides, spaced 1 millimeter between them, and treated with argon plasma irradiation. The exposed region of the substrate not covered by the glass is expected to acquire more wettable characteristics.

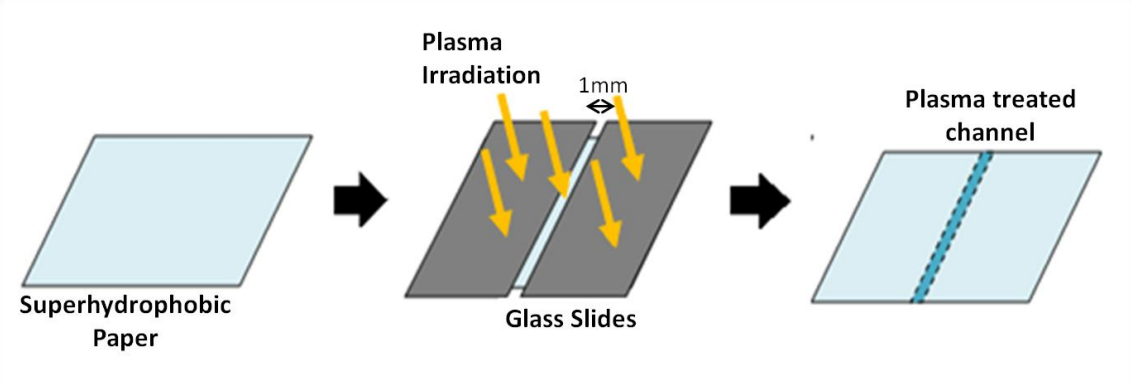

Figure III. 1 - Schematic representation of the patterning process of superhydrophobic paper. The glass slides limited the treatment area to channel-like regions.

2.2.3. Surface Characterization

The morphology and the topography of samples surface were analyzed by Scanning Electron Microscopy (SEM), using a JEOL JSM 820 microscope. The surfaces were sputter coated before with a thin gold layer.

The wettability of the prepared surfaces was evaluated by WCA measurements [28], using an OCA15+ goinometer from DataPhysics (Germany), at room temperature, with a conventional sessile drop method. A 5 µl droplet of HPLC grade water was deposited on the

surface. The shape of each droplet was recorded and analyzed using the SCA 20 software. At least three measurements were taken for each sample.

X-ray Photoelectron Spectroscopy (XPS) analysis was performed using a VG-Microtech Multilab 3000 spectrometer (VG Scientific) equipped with a hemispherical electron analyzer and an x- ray tube with Mg and Al anodes.

3 | Results and Discussion

With the immersion of the paper samples inside the chloroform solution containing PHB, the specimens started to soak, permitting PHB to penetrate into the paper fibers network. The immersion of these samples in the coagulation bath induced the precipitation of the hydrophobic polymer, observed mainly in the surface as a sphere-like aggregate structure (see Figure 2A and 2B). Moreover, it is possible to visualize some amount of PHB embedded between the cellulose fibers (see labels in Figure 2C and 2D), revealing that this hydrophobic agent stays attached to the entire substrate. The obtained structure, achieved after the modification with PHB, clearly contrasts with the fibrous texture of the original paper (see Figure 2E).

The protocol to induce PHB precipitation involved the mixture of a solvent and a non-solvent of PHB: chloroform and a mixture of ethanol and water, respectively. When we immersed the polymer solution into a coagulation bath a separation in two phases occurred: a PHB-rich phase and a PHB-poor phase [25, 29, 30]. As chloroform and ethanol are miscible [31], the solvent in the polymer solution is exchanged by the non-solvent (ethanol) and the PHB precipitation can occur [32]. Consequently, in the PHB-poor phase, polymer nuclei are generated by precipitation. Meanwhile, the PHB-rich phase aggregated around these nuclei, in order to decrease the surface energy [25, 33]. During polymer precipitation in the rich PHB phase, a continuous deposition of sphere like structures takes place (Figure 2B). Due to the low miscibility between the chloroform and the water (less than 0.8g/100 ml) [34], the presence of water in the coagulation bath acts like a selective barrier, avoiding the PHB spreading off the paper. However, the ethanol is highly miscible with both water and chloroform [31, 34] so the demixing liquid-liquid happens fast enough, inducing the precipitation of PHB.

The original paper presented a WCA of 110.4±0.8° (Figure 3A). Upon modification with PHB, the WCA rises up to 153.0±0.7° (Figure 3B), being clearly in the superhydrophobic regime.

To evaluate the robustness of the prepared superhydrophobic substrates we carried out a simple non-standard mechanical test with the finger [24], forcing it down the surface. Even supposing that the paper presents itself some brittleness, and for that inspires a careful manipulation, this trial reproduces the indispensable handling of the substrate: the WCA changes from 153.0±0.7° to 150.2±3.8°, indicating that the prepared substrates exhibit a good robustness behavior. The little discrepancy between the WCA's and the increasing of the standard deviations could be consequence of some accumulation of dust or oil in the surface, derived from the finger contact.

With further argon plasma treatment one can decrease the WCA of the superhydrophobic surfaces; using exposure times of the order of seconds one can reach hydrophobic surfaces (Figure 3C) but for higher exposure times it is possible to obtain superhydrophilic surfaces (Figure 3D). Upon modification with plasma we measured the WCA of the samples after different time points (1 day, 3 days and 5 days) and during this period no significant recovery of hydrophobicity was observed.

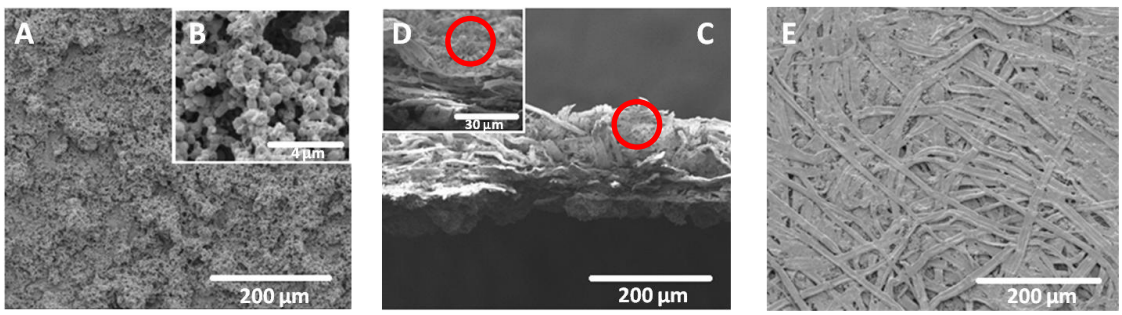

Figure III. 2 - SEM images of A) the surface of superhydrophobic paper and the corresponding magnification (B). C) The transversal fracture, obtained in liquid nitrogen, of the superhydrophobic paper and corresponding magnification (D). E) Representative SEM image of the commercial copy paper used to prepare the substrates.

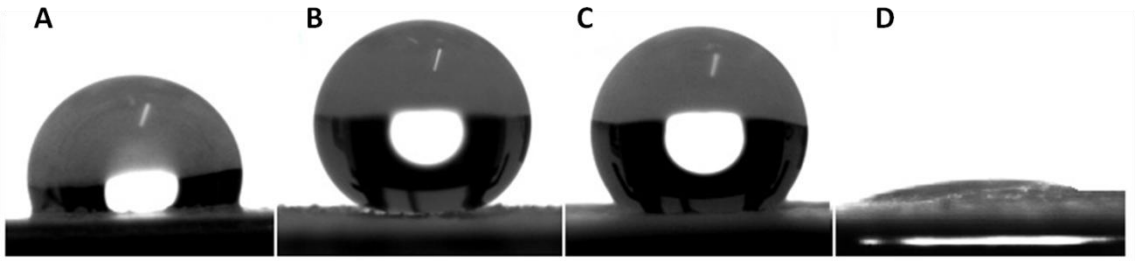

Figure III. 3 - Representative images of a 5 µl water droplet sitting over: A) commercial copy paper; B) superhydrophobic copy paper; Superhydrophobic paper treated with (C) 5 seconds (D) and 180 seconds of argon plasma irradiation.

The obtained superhydrophobic surfaces were treated with argon plasma from 5 to 180 seconds. Even for the lowest treatment time (5 seconds of plasma treatment), the WCA decreased down to the hydrophobic regime (141.0±1.7°) – see Figure 3C. With increasing treatment time the sample became clearly more wettable. As the substrate is based on cellulose, a material that permits some water swelling [24, 35], we investigated the stability of the deposited water droplets on the modified substrates: Figure 4A shows the time evolution of the WCA of the droplets of water deposited in surfaces previously subjected to plasma treatment for different times. For the unmodified superhydrophobic substrate and for exposure times of 5 seconds no changes in the WCA are detected with the contact time of the droplet with the surface. However surfaces with longer exposure time to plasma have enough wettability properties that permit the diffusion of water molecules inside the cellulose structure: after a certain time period, which decreases with increasing plasma treatment time, the region of the surface close to the water droplet gets more wettable and the measure WCA starts to decrease. Such phenomena could be explained by the disruption of the hydrogen bonds [36] within amorphous fraction of the cellulose fibers upon water absorption that renders the substrate more hydrophilic. The 5 seconds treatment is not sufficient to trigger this process. We can define an arbitrary threshold value of WCA below which the surface is clearly hydrophilic; Figure 4B shows the time necessary for a droplet deposited onto the surfaces treated with different plasma irradiation times to reach a WCA of 20°. For treatment times above 60 seconds the spreading of the water droplet is quite fast, being shorter than 20 seconds. Note that the same trend would be observed if other thresholds value either than 20° would have been chosen.

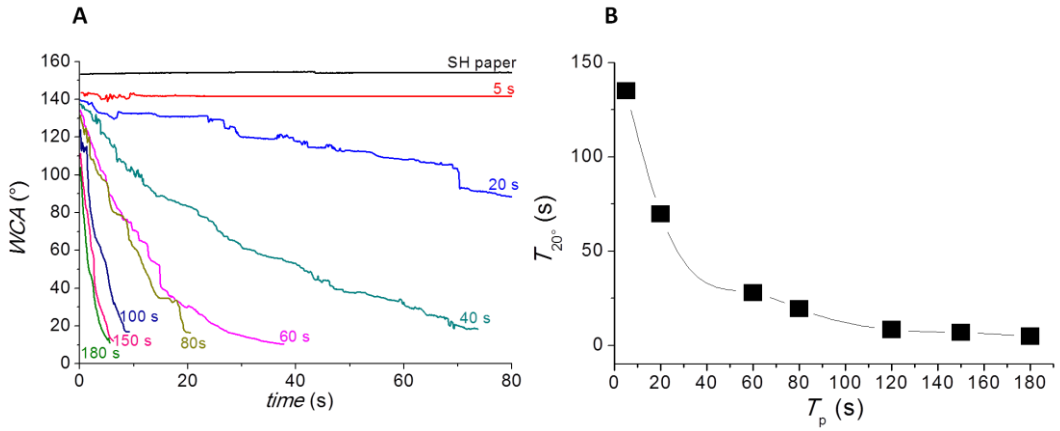

Figure III. 4 - Graphical representation of: A) evolution in time of the measured WCA of a 5 µl water droplet dispensed on surfaces of initial superhydrophobic paper treated with plasma for different times (in seconds); B) the time needed for the different water droplets to reach a WCA of 20 ° as a function of the plasma treatment time, *Tp.*

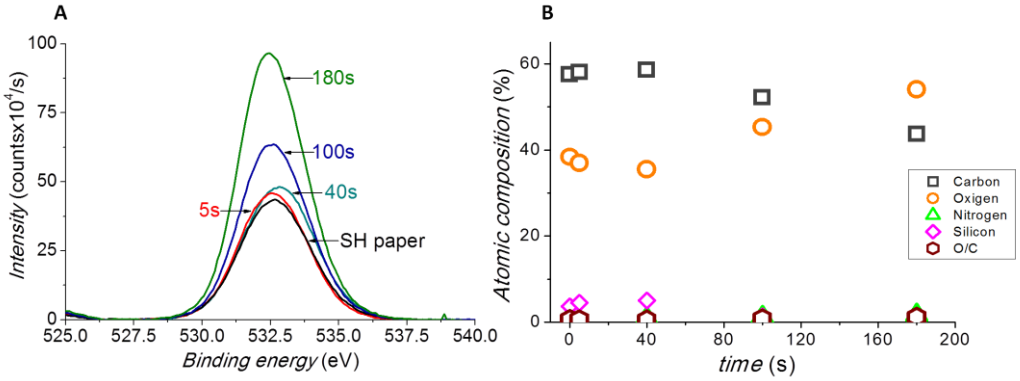

Figure III. 5 - A) O1s photopeaks for the samples under study and B) Surface atomic composition of samples treated with plasma for different exposure times.

The surface chemistry of the treated samples was analyzed by using XPS. Figure 5a shows representative O1s photopeaks. It is clear that there is an evident evolution of the XPS photopeaks for binding energies in the range of 525 to 540 eV, showing an increase of the oxygen content as a function of time. The surface composition of the samples was obtained from the XPS data. Figure 5b shows the results as a function of the treatment time. The non-treated sample is mainly composed by carbon and oxygen, but small amounts of silicon, as a contaminant, and nitrogen are also detected. For times of treatment around 100 seconds it is observed a significant and relative decrease in carbon and a noticeably increase in oxygen on the surfaces. In fact the O/C ratio increases from about 0.7 to around 1.3 for the sample treated for 180 seconds. The amounts of silicon and nitrogen keep very small for all the analyzed samples.

Therefore, the plasma treatment increased the amount of oxygen on the surface, increasing the quantity of C-O, C=O and COOR moieties [37]. This increment caused a significant reduction of both WCA value and the times to reach low WCAs [38] as it was observed in Figure 4. It should be noticed that the plasma treatment did not lead to appreciable changes in the topography of the samples, as seen by SEM (data not shown).

By controlling in space the exposure of argon plasma, it should be possible to pattern the superhydrophobic paper with more wettable regions. For the proof-of-concept we used two slide glasses to create an open channel shaped mask (Figure 1). By controlling the treatment time it was possible to create more wettable paths with the exposed geometry onto the superhydrophobic surface due to the oxidation of the uncovered area. Such patterned superhydrophobic substrates were proposed before as open-microfluidic devices, in which water could flow along the patterned channels without transposing the confined geometries, due to the high contrast in surface tension [25]. We chose 5 seconds plasma treatment to pattern the substrate because it is sufficient to create a contrast of wettability without changes in WCA with time (as it is observed in Figure 4A). We compared the behavior of the flow of water in different substrates: commercial copy paper, superhydrophobic paper and the open-channel patterned substrate treated with 5 seconds of plasma treatment – see Figure 6.

In the smooth paper surface – see Figure 6A - we can observe the increasing of the drop size as it advances over the leaning surface, spreading in both lateral directions, with no spatial control, due to the progressive absorption of water by the paper.

In the superhydrophobic surface the release of water droplets is hampered by the repellence of the substrate. As expected, the droplet roll off the surface – see Figure 6B.

For the channel-patterned substrate the drop had preference for the treated path, due to the fact that the patterned region is more wettable. Therefore, it was possible to retain and control the water flow along the confined geometry - see Figure 6C.

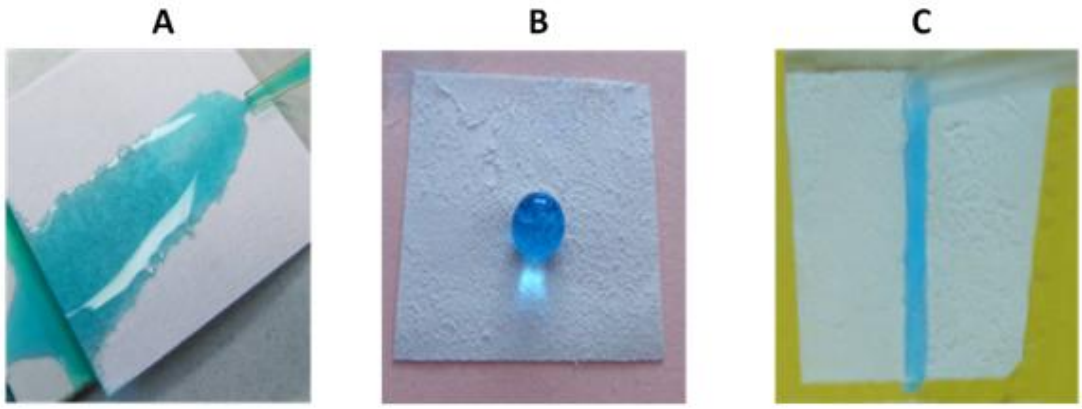

Figure III. 6 - Representative images of a 0.815 ml/min flow of blue colored water onto a A) commercial copy paper; B) superhydrophobic paper based substrate and C) open hydrophilic channel of 1mm of width patterned in a superhydrophobic paper substrate. All substrates were slightly leant so that the water could flow by the effect of gravity.

4 | Conclusions

This work proposed a new solution to fabricate superhydrophobic paper-based substrates, involving a simple phase separation approach, using a natural origin polymer (PHB). The results confirmed that the methodology is able to prepare robust, flexible, non hazardous and non wettable paper-based surfaces, which can be applied in different biomedical and environmental applications. The wettability of the obtained substrates can be controlled by adequate argon plasma treatment. Such surface modification capability provides a powerful tool to achieve easy, inexpensive and reliable substrates, which could be patterned with more wettable regions, just by exposing surface selected areas to an argon plasma treatment. The created paths permit the control of the water flow just using the effect of gravity.

5 | References

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CHAPTER IV.

SUPERHYDROPHOBIC PAPER IN THE DEVELOPMENT OF DISPOSABLE LABWARE AND LAB-ON-PAPER DEVICES

Chapter IV. Superhydrophobic paper in the development of disposable labware and lab-on-paper devices

SUPERHYDROPHOBIC PAPER IN THE DEVELOPMENT OF DISPOSABLE LABWARE AND LAB-ON-PAPER DEVICES

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Abstract

Traditionally on superhydrophobic surfaces history, the focus has frequently settled by the use of complex processing methodologies using non biodegradable and costly materials. In the lights of recent events on lab-on-paper emergence, there are now some efforts for the production of superhydrophobic paper but still with little developments and confined in the fabrication of flat devices. This work gives a new look over the range of possible applications of bioinspired superhydrophobic paper-based substrates, obtained using a straightforward surface modification with poly(hydroxybutyrate). As an end-of-proof of the possibility to create lab-on-chip portable devices, the patterning of superhydrophobic paper with different wettable shapes were shown with low-cost approaches. Furthermore, we suggested the use of superhydrophobic paper as an extremely low-cost material to design essential non-planar lab apparatus, including reservoirs for liquid storage and manipulation, funnels, tips for pipettes or accordion-shaped substrates for liquid transport or mixing. Such devices take the advantage of the self-cleaning and extremely water resistance properties of the surfaces as well as the actions that may be done with paper such as cut, glue, write, fold, warp or burn. The obtained substrates showed lower propensity to adsorb proteins than the original paper, kept superhydrophobic character upon EtO sterilization and are disposable, suggesting that the developing devices could be especially adequate to be used in the contact with biological and hazardous materials.

Keywords: Superhydrophobic surface; lotus-effect; biomimetic; patterned paper; lab-on-paper; paper labware; protein adsorption; Origami

1 | Introduction

Since paper origin, new ways of using it have been devised day-by-day. Today, the range of possibilities seems practically illimitable; indeed paper can be written, or printed but as well cut, folded, coated, embossed, colored, packed and so on. Thanks to its low-cost, biodegradability, wide availability and ability to modify and functionalized cellulose fibers, paper has been recognized as an appealing substrate to be applied also for scientific purposes [1-5]. Recent literature suggests their interest in microfluidic devices for biochemical analysis or less expensive health and point-of-care (POC) diagnosis [6-8], MEMS systems [9] and two-dimensional (2D) systems to transport, mix and storage liquid samples [10, 11]-

In particular, following the innovative studies of Whitesides' group [12-14], many attempts have been made to obtain patterned paper-based devices, most based on the same working principle: the contrast hydrophobic-hydrophilic [1].

For the past five years, many processing methodologies have been reported to develop patterned paper microfluidic devices, mostly exploring photolithography [12, 15], plasma treatment [16, 17] ink-jet printing [18] and wax printing [19, 20] approaches. However, little research has focused yet on superhydrophobic (SH) paper [21-24]. We recent suggested a simple methodology to prepare bio-inspired rough SH paper surfaces through a simple surface modification using poly(hydroxybutyrate) (PHB): the resulting substrates exhibited apparent water contact angle (WCA) of 153.0±0.7° [25]. The SH paper may bring new strengths on the development of lab-on-paper (LOP) devices: the increase of the wettability contrasts between wettable and repellent regions, the prevention of water and moist absorption and the avoidance of pathogenic contamination [22-24, 26].

This work proposes different methods to pattern flat SH paper surfaces creating wettable domains that contrast with the superhydrophobic behavior of the remaining area.

Currently, the design of LOP devices are mainly achieved by patterning techniques. Since Whitesides and their co-workers reported the use of photoresist agents activated by a UV light to pattern the surface of chromatographic paper with more hydrophobic domains [12], faster and straightforward techniques have been conceived. For instance, by using a standard inkjet printer Whitesides [13] achieved a more efficient route to pattern the paper substrate with regions with distinct wettabilities; Lu *et al.* [20] developed a microfluidic paper-based analytical device by drawing different patterned geometries with a wax crayon or with a printer, followed by a heating

step to melt the wax. We hypothesize that better results could be produced using SH substrates, creating a well-marked contrast with higher definition and increase the stability of the liquid dispensed in the wettable domains. It is widely known that processes such plasma treatment and UV irradiation are able to modify chemically rough SH surfaces, just by adjusting the exposure time [27, 28].

Three- dimensional (3D) paper-based systems are suggested in the literature: Martinez et al. [14] reported the fabrication of a multi-layered structure composed by single layers of patterned photo-resist paper stacked one above the other for biochemical detection; Liu and Crooks [29] developed an origami-inspired patterned paper which can be unfolded for parallel chemical analysis. Ge *et al.* [30] described a wax-patterned 3D paper based electrochemiluminescence immunoassay to detect tumor markers. In this work, we also intend to extend the use of paper to produce 3D devices by exploring the self-cleaning and extreme water repellent properties of the developed SH paper. In particular, we consider that SH paper would be profitable to reproduce conventional laboratory materials. Plastic (mainly polystyrene, polypropylene and polycarbonate) and glass materials are widely used by labware industry to produce beakers, eppendorf, disposable tips, pipettes and so on [31]. Effectively, there is a lack of low-cost and disposable alternatives to these materials, mainly for resource-limited laboratories. We hypothesize that SH paper, being flexible, inexpensive, safe, low carbon footprint and incinerable, could be an alternative material for such kind of applications.

2 | Materials and Methods

2.1. . Materials

The cartridge paper sheets, used for the development of paper-based substrates, were purchased from Pontus®. This type of paper distinguishes of common paper by its higher density (120g/cm^3), allowing greater resistance to handling. Commercial grade poly(hydroxybutyrate), PHB, with a melting point of 173 °C, was kindly provided by Biomer in the form of a very thin powder. Chloroform and absolute ethanol, with 99% and 99.5% of purity, were supplied by Carlo Erba Reagent and Pancreac, respectively.

2.2. Methods

2.2.1. Preparation of superhydrophobic paper

SH paper samples were prepared based on a process proposed before [25]. Paper samples with different sizes and shapes were cut from A4 sheets of cartridge paper and then immersed in chloroform for at least 6 hours, to extract any possible additives soluble in the solvent. The pieces of paper were then immersed for a period of 6 hours into a 7.5% (w/v) solution of PHB in chloroform. Subsequently, a new immersion was carried out into a coagulation bath, composed by a mixture of 85/15 (v/v) of ethanol and water, for 12 hours. The resulting samples were then placed in a glass surface and dried at room temperature and atmospheric pressure.

2.2.2. Characterization techniques

Scanning Electron Microscopy (SEM)

The morphology and the topography of samples surface were analyzed by SEM, using a Leica Cambridge S-360 SEM (Leica Cambridge, UK). The surfaces were sputter coated before with a thin gold layer.

Protein adsorption quantification – BCA assay

Through the use of a bicinchoninic acid (BCA) protein assay kit, supplied by Pierce Chemical Co (USA), we could quantify the protein absorption onto the surfaces of both original and SH paper by a simple colorimetric detection method. The operating principle is based on use of BCA to detect Cu^{1+}. In the presence of a protein and an alkaline environment Cu^{2+} is reduced to Cu^{1+}, providing a purple-like reaction. Cu^{1+} displays a strong absorbance at 562 nm, showing a linear behavior with the rise of protein concentration.

Circular samples (10 mm) of common and SH cartridge paper were carefully fixed on the bottom of an ultra low attachment 12-well plates and immersed in 3ml of five different concentrations of Bovine serum albumin (BSA, Sigma-Aldrich) in phosphate buffered saline (PBS, Gibco): 7 $\mu g\ ml^{-1}$, 16 $\mu g\ ml^{-1}$, 32 $\mu g\ ml^{-1}$, 78 $\mu g\ ml^{-1}$,150 $\mu g\ ml^{-1}$ and 260 $\mu g\ ml^{-1}$. The analysis was made after 1 hour of BSA adsorption. The remaining protein in solution was evaluated, according with the provider's instructions. The protein concentration was determinated by the

measure of the absorbance using a calibration curve for BSA, established using a series of protein solutions with known concentrations. The absorbance was read on a microplate spectrophotometer (Tecan), at 562 nm. The amount of protein absorbed was calculated for each sample subtracting the obtained value of microplate reader from the initial value of protein put in contact with the substrate.

Sterilization procedure

To entirely fulfill the requisites of general laboratorial material, the SH paper surfaces must be resistant to at least one sterilization technique. Squares of common, SH and patterned paper with 1 cm^2 of surface area were sterilized by ethylene oxide at Pronefro (Portugal). Standard conditions involve a working temperature of 45°C, a cycle time of 14 hours, a chamber pressure of 50 kPa and a humidity level of 50%.

Apparent Water Contact angle (WCA)

The wettability of the prepared surfaces was evaluated by WCA measurements, using an OCA15+ goinometer from DataPhysics (Germany), at room temperature, with a conventional sessile drop method. A 3 μl droplet of HPLC grade water was deposited on the surface. The shape of each droplet was recorded and analyzed using the SCA 20 software, 15 seconds after the drop deposition. At least five measurements were taken for each sample.

2.2.3. Patterning of two-dimensional (2D) superhydrophobic surfaces

In order to modify the wettability of the samples, we used a ProCleaner 220 (BioForce Nanoscience, USA) to treat the surface with a UV/ozone (UVO) irradiation. The exposure time ranged between 1 and 30 minutes. After the treatment, all the substrates were stored in a dry environment until characterization. Distinct geometries were patterned on the samples surface, emphasizing the multifaceted ability to create low-cost designs. For the UVO treatment we used acetate polymeric sheets that were cut with desirable hollowed geometrical features. By putting such masks over the SH paper, the cut-out part of the acetate sheet exposes confined regions of the sample to the UVO light, increasing the wettability in just these regions.

Also writing, printing and stick-on flat objects are here considered as innovative tools to modify and pattern the SH paper surface. We investigated simple water-based markers (Uso®), stick-on circles (Uso®) and printing models to pattern the substrates with hydrophilic domains. The printing models were developed in Word 2007® software and printed in a standard and commercially available Inkjet Printer (Epson Stylus SX105).

We used standard areas (9 and 16 cm^2) to prepare SH paper structures. The photos were taken by a Panasonic photographic digital camera (Lumix FS14).

2.2.4. 3D superhydrophobic paper architectures

Different structures were engineered using the developed SH paper, in order to reproduce some conventional labware, that can be obtained by basic origami or cut/glue methods.

2.2.5. Statistical analysis

All data are described as a mean ± standard deviation. Statistical significance was analyzed using the one-way ANOVA test, with $p<0.05$ considered differences as statistically significant.

3 | Results and Discussion

3.1. Characterization of superhydrophobic paper surface

SEM analysis

Changes in the topographical structure of paper surface using the proposed methodology were confirmed by SEM. Paper is composed by smooth cellulose fibers - see Figure 1A. PHB, dissolved in chloroform, was put in contact with paper surface, penetrating into the cellulose fibers randomly organized. When immersed into the coagulation bath, the non solvent started to diffuse into the polymer solution: as ethanol and chloroform are miscible, the exchanges between solvent and non solvent beyond the paper surface resulted in a thermodynamic instability of the system [32-35]. In order to attain the minimum Gibb's free energy, the system replies with phase separation of PHB, separating in both poor and rich polymer phases [32-34, 36]. Particular rough structures were generated; the precipitation of PHB-poor resulted in the disperse polymer

nuclei and the precipitated PHB-rich phase formed the polymer matrix, aggregating around these nuclei [37, 38]. The sphere-like nano-architectures may be a consequence of the continuous deposition of PHB, precipitated in the rich phase – see Figure 1B. Note that the water content of the coagulation bath avoided the PHB spreading of the paper fibers surface.

Common cartridge paper exhibited a WCA of 70.5± 2.51° whereas the obtained rough surface ascents to 155.8±2.84°- see representative images of water droplets over the surfaces on the top of Figure 1A and B, respectively.

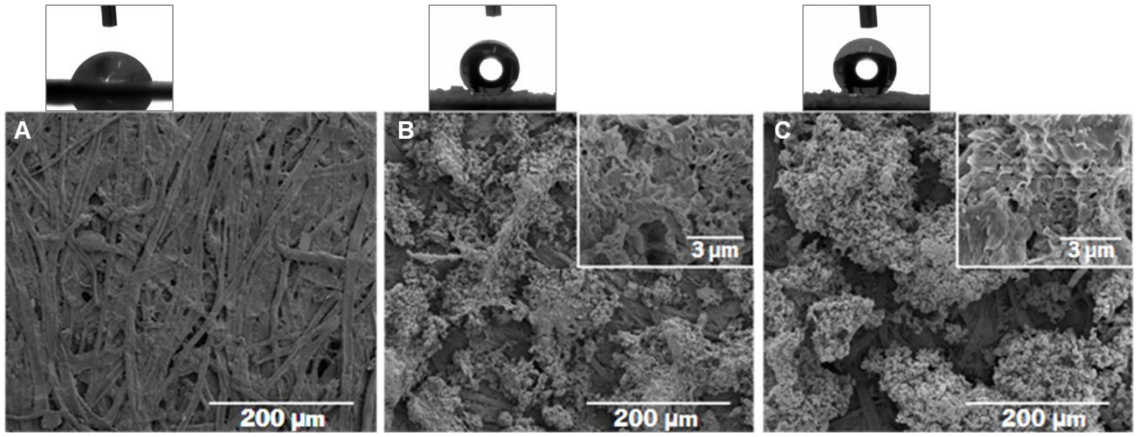


Figure IV. 1 - Representative SEM images of A) commercial cartridge paper used to prepare the substrates, B) the surface of SH paper and C) SH paper after ethylene oxide sterilization. The inset images are magnified SEM pictures. The top images show representative profiles of 3 µl water droplets over the surfaces.

Protein adsorption study

Low-protein adsorption materials are valuable in many areas (biosensors, surfaces that cannot be often cleaned, labware, medical textiles, food packing and so on) since they can reduce the biological contamination of surfaces [39, 40]. Previous works reported the influence of micro and nano topographies on protein adsorption [41, 42]; however just a small number of studies assessed protein adsorption on SH surfaces [39, 43-45]. We used BSA as a model biomolecule in the protein adsorption tests. As far as we know this is the first protein adsorption study performed with water repellent paper surfaces.

Figure 2 shows the amount of adsorbed protein on both regular and SH paper after being exposed to BSA solutions of different concentrations. The results indicate that: non wettable surfaces exhibit significant more resistance to protein adsorption than wettable paper surfaces, for the all range of initial concentrations explored. Such data are consistent with results previously obtained in other substrates [43, 44, 46] and may be explained with the Cassie and

Baxter model [47]. In fact, if we consider that the air remains in the lower topographical regions of the surface, it will be expected a decrease of the contact area between the surface and the protein solution.

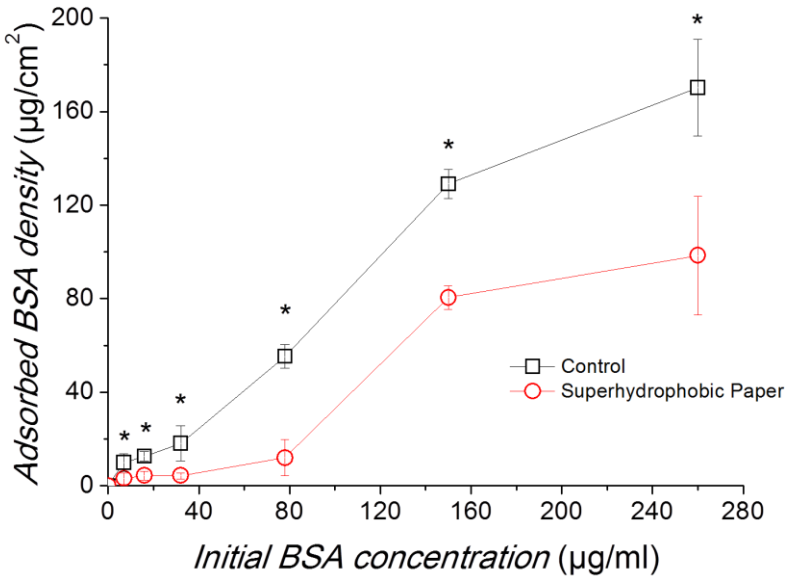


Figure IV. 2 - BSA adsorption after one hour of incubation in solutions with different initial protein concentrations, comparing: rough superhydrophobic paper circles and commercial cartridge paper squares. The inset graphics amplifies the results for the lower values of initial BSA concentration. *The differences for the same initial BSA concentration are statistically significant (p≤0.005).

Sterilization effects

Lab-on-chip devices and labware often require to be sterilized, after which their properties should be preserved [48]. To evaluate the pos-sterilization behavior using ethylene oxide, the surface topography of SH paper was analyzed by SEM – see Figure 1C. No significant changes are detected when compared with the non-sterilized SH paper (Figure 1B). The WCA of pos–sterilized SH paper samples was 151.8 ±1.81˚, indicated that sterilization did not compromise the SH behavior of the substrate.

Wettability modification

WCA data reveals the decrease of wettability of SH paper samples, at different exposure times to an UVO irradiation – see Figure 3. As the time of treatment increases, the surface become more wettable; for 30 minutes of UVO exposure the SH paper surface is switched to a very hydrophilic stage (WCA= 11.4±3.5˚). Upon modification with UVO we measured the WCA of

the samples after different time points (1 day, 5 days and 20 days) and during this period no significant recovery of hydrophobicity is observed.

The modified surfaces using UVO irradiation for 8 minutes was subjected to ethylene oxide sterilization. No significant differences could be detected in the WCA with the non-sterilized sample. Figure 4 shows the influence of sterilization in the WCA for the original paper, SH paper and the SH paper irradiated with UVO, that evidences that ethylene oxide sterilization has a low influence in wettability. This is in a good agreement with literature: ethylene oxide is described as a non aggressive technique for a wide range of polymeric materials, with no significant change in their properties [49].

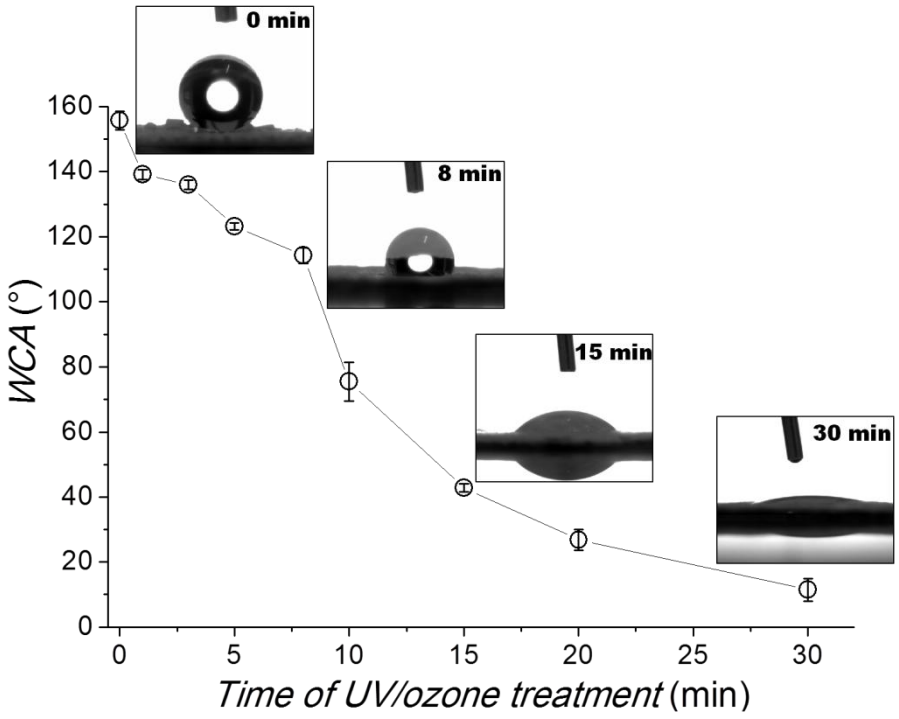


Figure IV. 3 - WCA of a 3 µl water droplet dispensed on initially SH paper treated with UVO irradiation for different times (in minutes). Micrographs showing the profiles of 3 µl water droplets over the surfaces are shown for specific time-points.

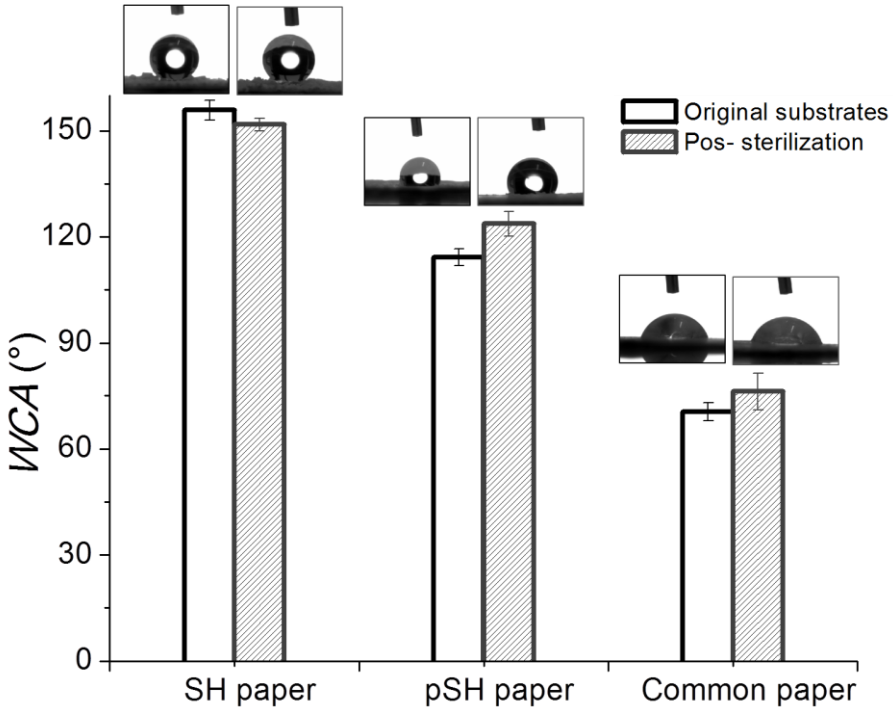


Figure IV. 4 - WCA of 3 µl water droplets dispensed on different substrates: comparison between non sterilized and ethylene oxide sterilized SH paper, SH paper treated with 8 minutes of UVO irradiation and commercial cartridge paper. The graphic contains the picture of the profiles of 3 µl water droplets over the surfaces, for each case.

3.2. Creation of simple 2-dimensional LOP devices

As extensively reported by literature, the design of LOP devices are often based on the possibility to create different confined geometries onto the paper surface for fluidic flow or deposition of small liquid volumes [1, 4, 50]. This is generally achieved by patterning the surface with more wettable domains. We demonstrate the possibility to pattern the SH paper surface using distinct approaches, each one with the proper advantages- see Figure 5.

Using acetate sheets with hollowed geometrical features it is possible to expose defined wettable domains of SH paper to UVO irradiation (Figure 5A). We choose the UVO treatment for 8 minutes to pattern the substrate because this irradiation time is sufficient to create a contrast of wettability but still prevents liquid penetration into the substrate. For the proof-of-concept we created standard geometries onto SH paper substrates, which are revealed when we dispensed colored water in the hydrophilic spaces – see micrographs in Figure 5A. The UVO treated domains have well defined limits and confine very efficiently the water volumes within the corresponding geometrical shapes.

Easier-to access and faster approaches were also investigated to pattern wettable regions over SH paper based on the fact that paper may be painted, glued or printed (see Figure 5B).

We first used a water-based marker to draw and paint the desired pattern geometry onto the surface of the SH paper substrate- Figure 5BI. Also small paper stickers were glued to specific spots of the sample (Figure 5BII). With an easy-to-get marker or paper stickers is possible to coat single parts of the substrate with more wettable characteristics: WCA= 76.4±1.50° for water-based marker and WCA =82.9±4.28° for paper stickers. Such elements provided the ability to confine liquid volumes with a controlled geometry – see micrographs in Figure 5B. Both patterning processes involve straightforward bench-top methodologies and inexpensive resources [20].

For more complex patterning designs we propose an inkjet printing process. We started to produce a pattern model in a computer and printed it directly on a SH paper piece, using standard colored inks. The WCA of the printed areas is 76.4±8.20° and the resolution of the regions is defined by the specifications of the ink-jet printer. After printing, the patterned SH paper is ready to be used and the confinement of liquids is consistent with the shape of the modified regions. Figure 5BIII shows an example of printed channels that could be used to produce substrates for open microfluidic applications [27]. The good definition of the printed wettable domains, the simplicity and fastness of the procedure offer the possibility to produce at large scale low-cost LOP devices.

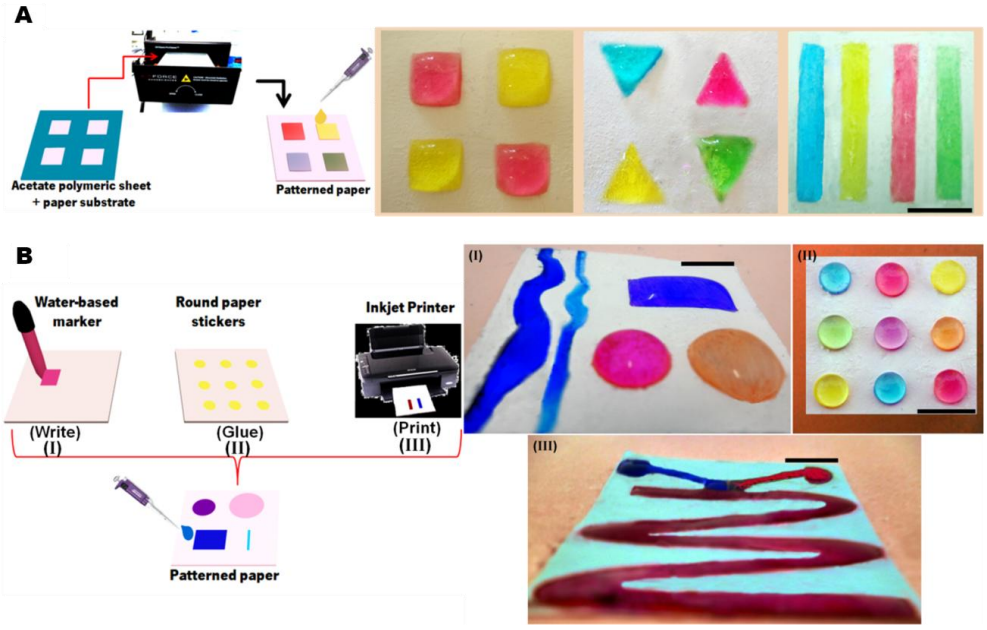


Figure IV. 5 - A) Schematic representation of UVO patterning to create more wettable domains on flat SH substrates and the respective representative images with colored water; B) Schematic representation of handling and printing patterning to create more wettable domains on 2D superhydrophobic substrates and the respective representative images of patterning with (I) water-based marker, (II) round paper stickers and (III) inkjet printer, revealed by colored water. The scale bars are 10 mm.

3.3. Construction of 3-dimensional superhydrophobic paper labware

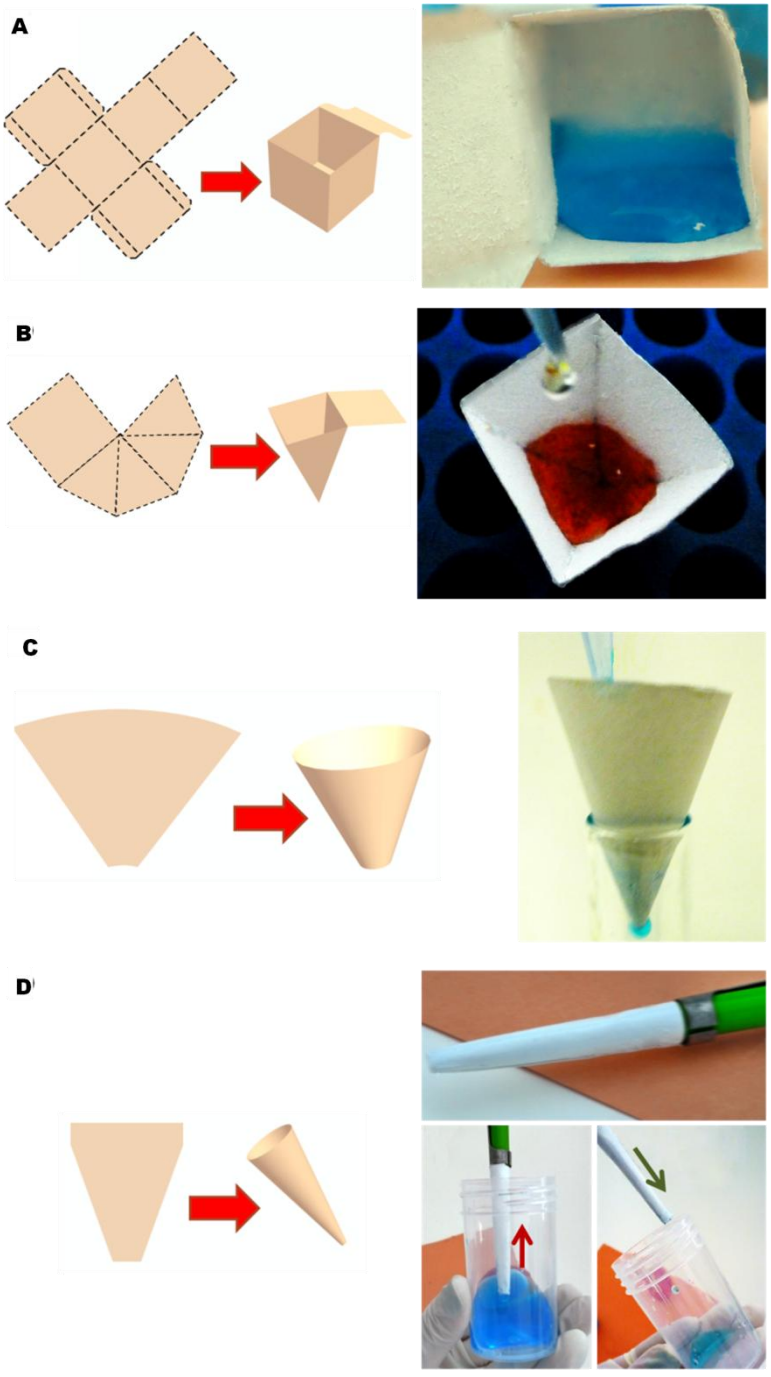
The produced SH paper can be shaped, cut, folded, creased, wrapped, glued to form free-standing 3D architectures. As far as we aware, this is the first time that SH paper is suggested for 3D constructs. Low cost and disposable materials are interesting for labware industry. We propose different designs to alternative low-cost labware that take advantage of the self-cleaning properties and water resistance of the developed material.

We describe two different structures, based on the folding of the SH paper substrates: a cube, which can be used in storing, transferring and mixing biological or chemical samples (see Figure 6A) and an eppendorf-like object able to perform the same function as plastic ones, even when exposed to centrifugal forces (Figure 6B). These materials can be produced in a wide range of sizes and can be easily written to identify the samples. The respective figures reveal the behavior of colored water in contact to the 3D assemblies: there is no adsorption of the sample at least for 24 hours.

Wrapping and glue SH paper also offers the possibility to fabricate useful structures in a simple way. Figure 6C shows a paper funnel with a SH inner surface, which can be used to transfer liquids. We dispensed colored water solution onto the object surface to verify its behavior. After continuous reuses the inner face of the funnel was able to retain its ability to repeal water, suggesting that it can be used repeatedly to transfer liquids without the need of cleaning procedures. Micropipettes are a daily used tool in laboratory procedures, using plastic tips to collect the liquid sample. We develop a SH paper tip, just by rolling up the substrate- see in Figure 6D. Paper porosity can be overcome blocking the pores, using a polish. Therefore the vacuum action provides the required force to draw up precise measures of liquid, defined by the user, to be transferred and dispensed it to a target place. Again, the self-cleaning and the low protein adsorption of the SH paper permit to extend the reutilization of the tips, before being dispensed. Additionally, its low-cost and the possibility to produce at large scales suggest that SH paper tips could be an alternative to traditional plastic tips.

Inspired by the accordion shape, we use a zigzag folding of a single piece of SH paper to create one or more confined divisions or channels. When we dispense different liquid solution into these channel-like structures the drops have tendency to roll off easily the surface till the end of the device- see Figure 6E. Such system may be useful to transport different liquids through

distinct channel compartments, and can also be used to promote their mixture in the end, if a fan-like architecture is adopted.



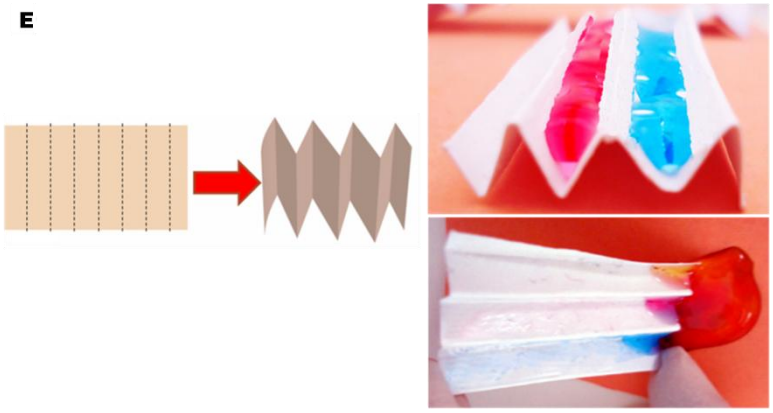


Figure IV. 6 - A) Schematic representation of 3D SH paper cube to transfer, mix and store liquid samples and the respective representative image when dispensed colored water; B) Schematic representation of 3D SH paper-like eppendorf and the respective representative image when dispensed colored water; C) Schematic representation of 3D SH paper funnel and the respective representative image when dispensed colored water; D) Schematic representation of 3D SH paper – like tips and the respective representative images of the tip inserted in P1000 micropipette, the drawing up of the liquid solution and the transference and dispensing on the target place; E) Schematic representation of 3D SH paper folded with an accordion shape for parallel liquid transfer and the respective representative images of the different channels covered by colored water solutions and the releasing of the liquids. The 3D superhydrophobic structures have the following volumes, respectively: 6 cm^3, 2.25 cm^3,3.14 cm^3 and 0.5 cm^3. The accordion has 16cm^2 of projected area and around 0.5 cm of height.

Especially when dealing with hazardous chemicals and biological liquids, substrates for diagnostics and on-site detection or for labware applications should be disposable after their use. Paper is a well known biodegradable compostable material. Moreover it can be readily eliminated by burning. Figure 7 shows that the developed SH paper may be easily incinerated up to an ash residue.

Figure IV. 7 - Representative image showing the possibility to incinerate the SH paper substrates up to an ash residue.

4 | Conclusions

In conclusion, we have made use of different abilities of paper to create useful 2D and 3D structures based on SH paper. By tuning the wettability of confined geometries of the surface to hydrophilic, we developed simple and low cost procedures to pattern the SH surface with well defined wettable regions; just by writing or printing we can produce a desirable patterning in a time scale of seconds or minutes. These results constitute an important step toward the development of completely flat LOP devices and substrates to manipulate liquid drops. SH paper substrates were also shown to be suitable to construct 3D structures with promising applications by fixing the geometry using glue of origami-based methodologies; we exploited the building of flexible, extremely low cost and disposable alternative labware, providing the framework for a new way to use SH paper. Instead of acting like a final and target application, our results provide a springboard for the development of a wide range of new disposable LOP and labware devices.

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CHAPTER V.

PATTERNED SUPERHYDROPHOBIC PAPER FOR MICROFLUIDIC DEVICES OBTAINED BY WRITING AND PRINTING APPROACHES

PATTERNED SUPERHYDROPHOBIC PAPER FOR MICROFLUIDICS DEVICES OBTAINED BY WRITING AND PRINTING APPROACHES

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Abstract

This work outlines inexpensive patterning methodologies to create open-air microfluidic paper-based devices. A phase-separation methodology was used to obtain biomimetic superhydrophobic paper, hierarchically composed by micro and nano topographies. Writing and printing are simple actions that can be used to pattern flat superhydrophobic paper with more wettable channels. In particular, inkjet printing permits to control the wettability of the surface by changing the darkness of the printed regions. The difference between capillary forces provides the possibility to control and drive liquid flows through the open path lines, just by titling the piece of paper. Additionally, maintaining a continuous flow, it is possible to direct the liquid at different volumetric rates in a horizontal position along non-linear channels printed/written over the surface.

Keywords: superhydrophobicity; biomimetic; patterned paper; open-air microfluidic devices

1 | Introduction

Superhydrophobic (SH) surfaces are a widespread field in scientific research. By definition, these surfaces exhibit extreme tendency to repel water droplets and are characterized by an apparent water contact angle (WCA) higher than 150° [1, 2]. Non-wettable surfaces were firstly founded in nature with the observation of biological systems: the lotus leaf is the archetypal example of superhydrophobicity [3-5]. Besides non wettable behavior, water droplets can also easily roll off on the lotus leaf surface, presenting a contact angle hysteresis lower than 10° [1, 2]. Barthlott and Neinhuis [4, 5] reported the nano and micro hierarchical organization of the surface topography of lotus leaf as principal leading factor of superhydrophobicity. Henceforth, a high number of studies have demonstrated that to produce artificial SH surfaces it is necessary to fulfill two requests: roughness topography and low surface energy [3, 6, 7]. Paper is essentially composed by fibrous cellulose. The hydroxyl groups of this polysaccharide turn the material hydrophilic [8]. The modification of paper to render the surface more hydrophobic, or even superhydrophobic, permits to extend its applicability. The increase of water resistance could be profitable to use paper in devices able to sustain the flow or the presence of water over the surface, such as in microfluidic applications.

Microfluidic devices have been largely reported for biomedical uses [9, 10]; the reduction of consumption of expensive reagents and the high surface-to-volume ratio are some of the most reported advantages. Different purposes have been suggested for these devices such as cell based-biosensors [11] and biochemical analytical systems [12]. Unlike traditional closed-microfluidics, open-air microfluidics (devices that do not present a roof), can overcome problems like the bubble trapping, the impossibility to access directly the surface and the complex fabrication techniques employed [10, 13]. There are different strategies to confine the liquid flow that are able to be used in the microfluidic manufacturing; one of the most advantageous is the surface-tension driven force using a selective hydrophobic/hydrophilic contrast [14, 15]. Different processes can be applied to obtain structured surfaces exhibiting patterns with different wettabilities; for instance Gau *et al.* [16] developed a open microfluidic device patterning a hydrophobic silicone rubber or a thiolated gold substrate with more wettable regions, using a thermal vapor deposition of magnesium fluoride (MgF_2) through appropriate masks. Progresses in microfluidic devices have been achieved by adapting the special features observed in lotus leaf [17-19]: Oliveira *et al.* [20] suggested the fabrication of open microfluidic devices through the

surface modification of a patterned SH polystyrene; Xing *et al.* [13] reported an unconventional droplet-driven system on a SH patterned polydimethylsiloxane (PMDS) surfaces by a lithographic methodology. The use of paper to produce such kind of devices could bring new advantages such as price and availability. The possibility of writing and printing on paper permit to change the local wettability in a sub-millimetric scale range.

Among recent works, inkjet and wax printing are the most common methodologies employed to pattern the paper surface [21-23]. Droplet handling and controlling operations using SH paper substrates were firstly suggested by Balu *et al.* [24] ,through printing hydrophilic patterning domains. However, such processes were never been proposed to use patterned SH paper in the area of microfluidics. Using a simple phase separation process to fabricate SH paper substrates [25], we present straightforward methodologies to modify the resulting substrates to fabricate inexpensive open-air microfluidic devices.

2 | Experimental work

We used a 7.5% (w/v) poly(hydroxybutyrate) (PHB) (Biomer) in chloroform solution to soak pieces of commercially available cartridge paper (Pontus®), previously treated with chloroform (Sigma-Aldrich) to remove any additives. After 6 hours, the PHB-soaked paper surfaces were rapidly immersed in a coagulation bath composed by 85/15 (v/v) of ethanol (Pancreac) and water during 12 hours. In the end, the samples were dried at room temperature.

Wettability measurements were performed with an OCA 15+ goniometer from DataPhysics (Germany), using the sessile drop method. The shape and the WCA values were recorded using the SC20 software, fifth seconds after depositing a 3 µl water drop (HPCL grade) onto the samples surface. At least five measurements were carried out for each condition. All results are presented as mean as standard deviation. The surface morphology was accessed by scanning electron microscopy (SEM) analysis, using a Leica Cambridge S-360 SEM (Leica Cambridge (UK)). All samples were pre-coated with a thin conductive layer of sputtered gold. Different magnifications were used with an accelerating voltage of 5 kV.

We wrote different paths onto SH surfaces: we used coal pencils and water based markers, purchased from UNO®, and a commercially available Inkjet Printer (Epson Stylus SX105). To study the liquid behavior onto the patterned superhydrophobic substrates we

dispensed colored water solution (a couple of hundreds of microliters) onto the open-channels/paths domains and carefully tilted the substrate. Additionally, different volumetric flows of colored water were also pumped along hydrophilic channels patterned on the SH paper surfaces, using a peristaltic pump (Ismatec). These substrates were patterned using the Inkjet printer, with a mid intensity color, and shaped as curved open-lines.

The representative images were taken with a Panasonic photographic digital camera (Lumix FS14).

3 | Results and discussion

During the modification of the surface of paper with PHB the thermodynamic of the system (PHB solution and coagulation bath) was destabilized by the mass transfer occurring on the interface, between the solvent and the non solvent [26-28]. This triggered a liquid-liquid demixing and the consequent polymer separation, forming both poor and rich polymer phases; the PHB-rich phase aggregates around the polymer nuclei precipitated in the PHB-poor phase [29, 30]. This leads to the formation of PHB asperities distributed in all the surface of paper – see Figure 1A. In the magnification of Figure 1A is possible to distinguish some sub-micrometer sphere-like structures deposited on the surface. The rough structure of SH paper, hierarchically organized at nano and micro-scales is quite distinct from the smooth fiber structure of the original commercial cartridge paper - see Figure 1B. The insets of the figures reveal a WCA of 155.8± 2.84˚ for the rough paper surface and 70.5± 2.51˚ for the original paper. The SH paper presents a contact angle hysteresis of 1.1± 0.10˚. The low adhesion of the water droplets to the SH surfaces suggests that the Cassie-Baxter model would be the most adequate to explain the repellency properties of the obtained substrate. Following this model, we consider that the liquid is suspended by air pockets, which are trapped between the rough structures [31]. The area fraction of the liquid-solid contact is given by:

$$f = \frac{\cos\theta^* + 1}{\cos\theta + 1} \qquad \text{(Equation V. 1)}$$

Where θ^* is the WCA of the rough paper surface and θ is the WCA of smooth PHB surface. Taking θ = 87.0˚ we can estimate f = 0.084.

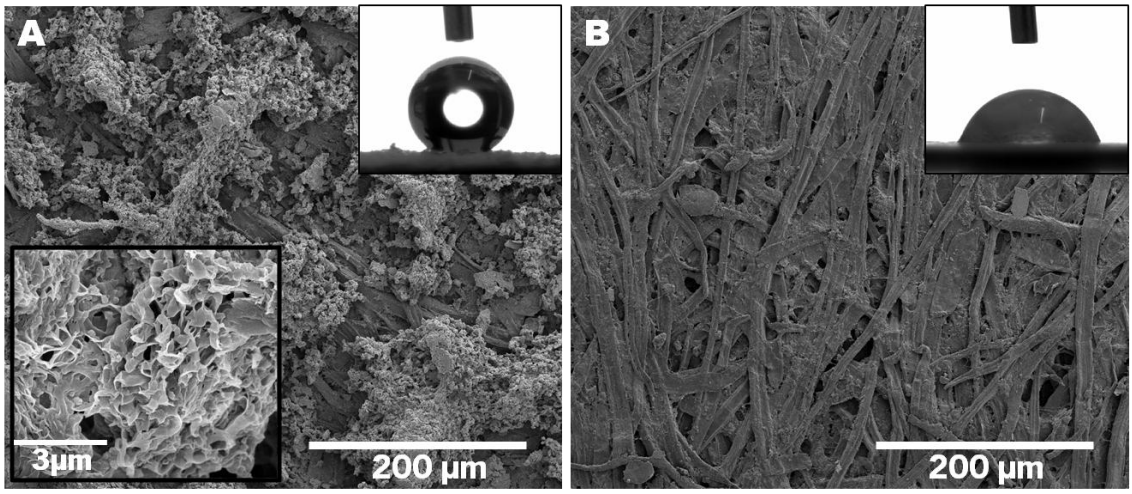

Figure V. 1 - Representative SEM images of A) SH paper surface and the corresponding magnification and B) commercial cartridge paper used to prepare the substrates. The inset images show representative profiles of 3µl water droplets over the surfaces.

To create wettable open-air channels on the SH paper we developed simple methods to pattern the surface, based on the writing possibilities offered by paper. Figure 2 presents the WCA of the different strategies and the correspondent insets show the representative images of the interfacial drop-surface contact. The wettabilities of the written areas are able to create a significant contrast with the surrounding SH areas.

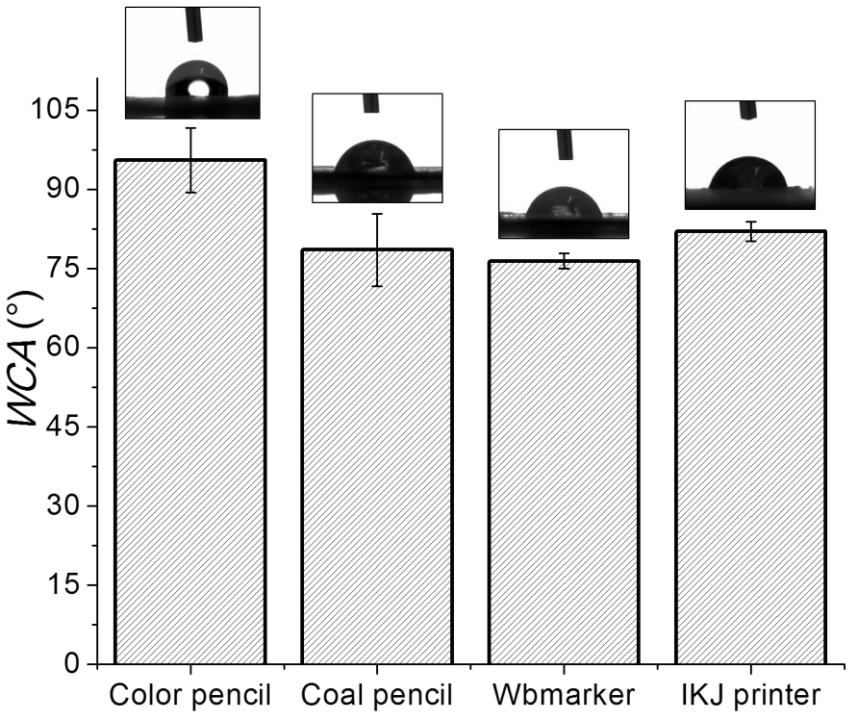

Figure V. 2 - WCA of a 3 µl water droplet dispensed on the different patterned domains of SH paper-based substrates with the corresponding representative profiles.

Figure 3 shows representative images of the open lines written with different shapes and widths. When colored water flows along the channel, the liquid is constrained between its limits. In Figure 3A it is possible to distinguish liquid flows along complex patterned paths with different widths: the width of the stream increases with the width of the printed line. The more acute the curves of the path are, the higher is the possibility of the liquid crosses the limits of the written line. The definition of the water stream is within the millimetric scale range. The irregularities that are detected should be related to the presence of graphite microparticles that are released outside the main lines during the writing. Such residues are hydrophilic and contribute for the irregular wetting observed. For the patterning with a water-based marker, we tested the behavior of the liquid onto a branched-like geometry. This process enabled again to increase the wettability of the written regions (Figure 2). As a consequence the liquid flows along the patterning without wetting the remaining area – Figure 3B. Comparing both cases it is to be noted that while the WCA correspondent to the patterning with coal pencil remains constant over the entire drop-surface contact, the WCA of the patterning with the water-based marker decreases gradually. This may explain the better results observed in second method. We believe that the no need of masks or specific equipments, the simplicity, the fastness, the energy-saving and the low cost are important advantages when comparing with traditional patterning techniques.

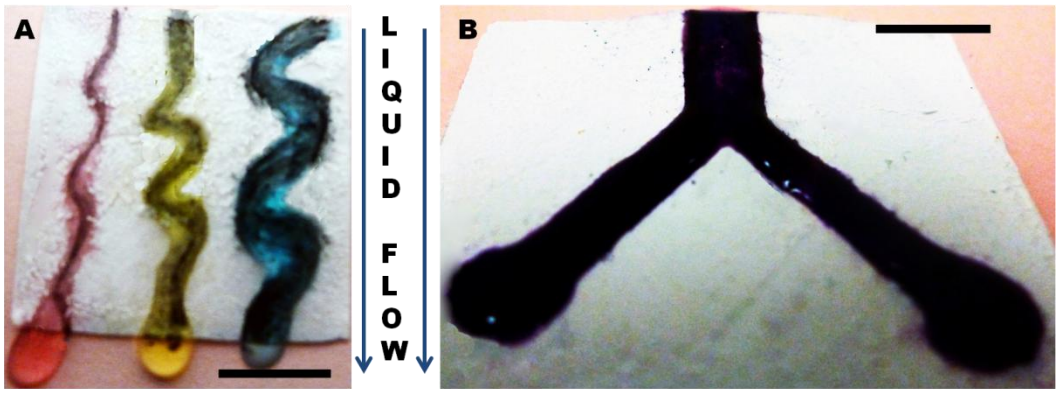

Figure V. 3 - Representative images of colored water flowing along wettable lines: A) curved lines written with coal pencil onto a SH paper substrate; B) branched line written with water based marker onto a SH paper substrate. The scale bars are 10 mm.

Inkjet printing has already been described in literature as suitable to create patterned paper surfaces [21, 32-34], mainly using solutions, solvents and proteins as modifier agents. We extend in this work this simple approach to print SH paper, that after modification presents a WCA of 82.0 ± 1.89° upon patterning with a mid color intensity. The liquid ink is distributed at

small volume rates, through a piezoelectric material in an ink-filled reservoir behind a nozzle. The piezoelectric changes the form when a voltage is applied to the equipment, providing a pressure in the fluid able to propel it from the nozzle. We used solvent-inks, where the main ingredient is a volatile organic compound; due to the solvent evaporation is possible to deposit conveniently the ink on the SH surface. Using different color grades, from lightest to darkest ones, it is possible to control the wettability: with the use of darken colors the surface becomes more wettable – see Figure 4.

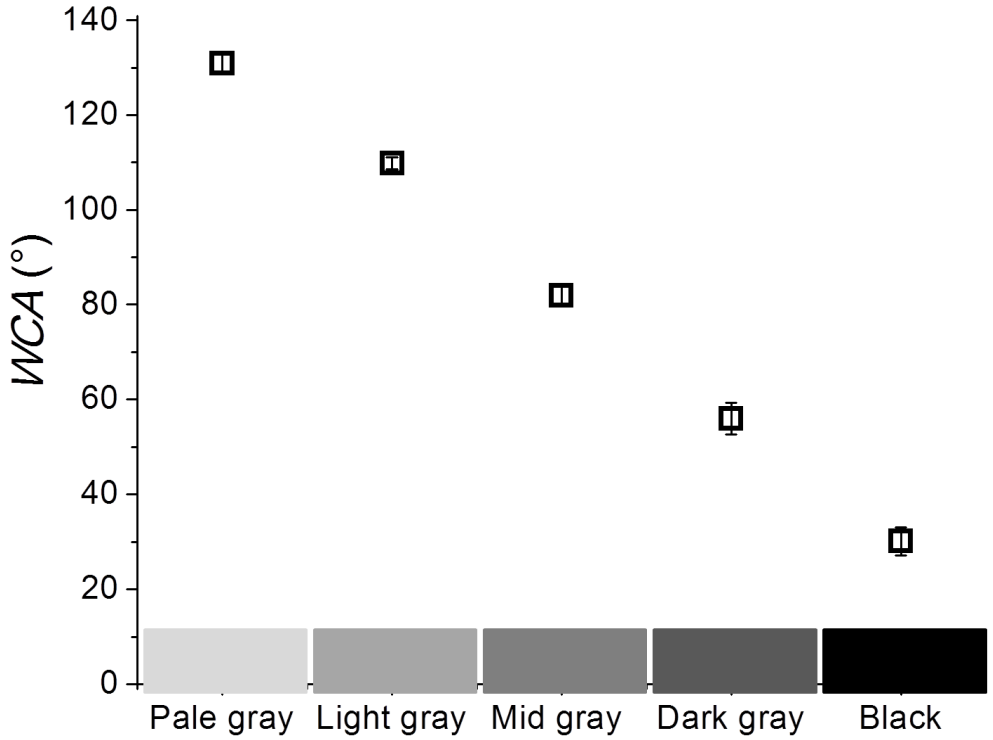

Figure V. 4 - WCA of 3 µl water droplet dispensed on printing patterned domains of SH paper-based substrates, with different intensities of color, and the corresponding representative profiles.

We compared the liquid flow on straight lines (Figure 5A) and curved lines (Figure 5B), induced by the effect of gravity. It can be seen that even with the curved lines, the liquid can follow reasonably the patterned paper. Additionally, we investigated the ability of the patterned SH paper substrates to enable different volumetric flows moving along a curved hydrophilic path. In this case the flow of water was controlled by a peristaltic pump. For a flow of 0.6 ml/min (Figure 6A), or even 1.6 ml/min (Figure 6B), the liquid follows the wettable path. For a volumetric flow equal or higher than 2.5 ml/min we had no longer a good spatial control over the liquid flow – see Figure 6C. All the cases above mentioned were achieved printing lines of 0.5

mm of width. We applied a 1.6 ml/min volumetric flow onto a hydrophilic line with 1.5 mm of width; as expected, the control of the liquid flow increases with the increase of the line width (in real, the real volumetric flow decreases when the width of the flow on the surface is bigger) – see Figure 6D. We developed low-cost and fast processes to pattern flexible paper substrates with good spatial resolution to produce open-air microfluidic devices.

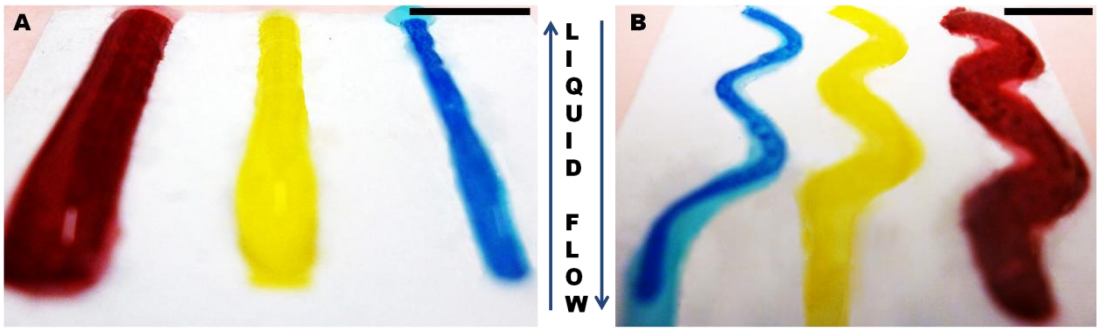

Figure V. 5 - Representative images of colored water onto wettable open A) straight curved lines and B) curved lines printed with an inkjet printer onto a SH paper substrate. The scale bars are 10 mm.

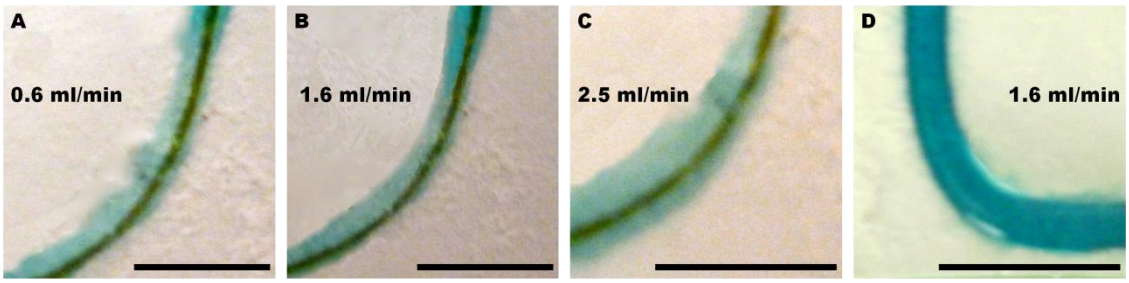

Figure V. 6 - Streams of colored blue water moving along a hydrophilic channel patterned on a SH paper surface, with different volumetric rates (ml/min): A) 0.6 ml/min, B) 1.6 ml/min and C) 2.5 ml/min; D) Stream of colored blue water moving along a larger hydrophilic channel patterned on a SH paper surface. The scale bars are 10 mm.

4 | Conclusion

Writing and printing are simple and effective tools to pattern SH paper substrates with more wettable domains. For the particular case of using inkjet printing it is possible to control the wettability of the printed regions by playing with the intensity of the deposited color. By writing or printing wettable lines it is possible to drive and confine a liquid flow along the predeterminated path. These findings add a growing body to the development of low-cost, straightforward and fast patterning techniques to microfluidic industry, namely for open-air microfluidics. This could

extend the use of paper in the development of new devices for biological and biomedical diagnostics/analysis.

5 | References

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CHAPTER VI.

GENERAL CONCLUSIONS AND FUTURE RESEARCH

1 | General Conclusions and Future Research

This project investigated the fabrication, characterization and further application of a low-cost superhydrophobic substrate, starting with a simple piece of paper. This research was undertaken to exploit the diverse advantages of using paper for the development of inexpensive biotechnological devices and to open new doorways in the microfluidics field.

Biomimetic rough paper substrates were obtained by a simple phase separation methodology, using PHB. Different characterization techniques were used to confirm the large increase of WCA after the modification with the PHB: the micro and nano structures resulting from the polymer precipitation, visible by the SEM, are directly related with the superhydrophobic properties assessed by WCA measurements. We hypothesize that such materials could find applicability in the biomedical and biological fields. Therefore, it was important to perform a protein adsorption study to complement the characterization of the SH paper substrates. We were able to conclude that the amount of adsorbed BSA was much less for rough SH paper surfaces, when compared with the original paper. This was explained using the Cassie-Baxter model, where the rough topography of the surface significantly reduced the liquid-surface contact. Other parameters such as the robustness of the surfaces and the ability to maintain their properties after ethylene oxide sterilization were also evaluated; the substrates exhibited resistance to manual handling and to EtO sterilization.

The characterization of the developed SH paper substrates was essential to develop 2D and 3D structures for different applications; we contemplated lab-on-paper (LOP), open microfluidics and labware devices. We started with a SH surface, which allowed us to develop LOP and open microfluidics devices, with defined patterned wettable regions at the millimetric length scale permitting to achieve high contrast of wettability. Distinct strategies to create patterned paper platforms were already reported by different groups. We applied different techniques to modify the wettability of confined geometries/paths on SH surfaces: plasma treatment, UVO irradiation, writing, gluing and printing approaches proved to be good alternatives to the conventional ones. Low-cost, simplicity and efficiency are advantages shared by these methodologies. Furthermore, writing and printing techniques can be conceived outside of the laboratory with readily available materials, asserting themselves as especially suitable for resource-limited laboratories or markets. Devices to manipulate, mix, handle or transfer/transport liquid solutions, tips and shaped structures to transport aqueous-based liquids

through distinct channel compartments were suggested to create an effective alternative to plastic and glass ones.

Research on paper-based devices is still at an initial stage and the modification of paper to a superhydrophobic state seems to be a very powerful tool to strengthen the scientific value of these substrates; biomedicine and biotechnology are the major target fields.

Further studies must be undertaken in order to characterize the patterned areas achieved by writing and printing methodologies, as well as for the validation of the treatment over a larger time periods. The developed approaches will provide the base for the development of increasingly cheaper point-of-care devices, through the functionalizing of the patterned areas with specific indication reagents. Additionally, patterned SH paper substrates can be interesting for combinatorial analysis of liquid drops or even smaller volumes, operating as a chip.

The design and the development of non planar SH paper structures will definitively challenge the labware industry; even with less mechanical resistance than glass or plastic, paper offers a lot of advantages such as the disposability and low-cost. Future studies are required to understand the SH paper possible limitations in every-day lab activities.

An interesting issue for further investigations is the study of the applicability of SH paper labware on biological assays; for instance, bacterial contamination tests onto the SH paper surfaces is an assay that should be performed.