

Rethinking the role of superhydrophobicity in non-cell-adhesive biomaterial surfaces.

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*Content: Bachelor Thesis*

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*Date: 08-05-2014*

Abstract.

The potential of superhydrophobic surfaces have gained increased interest in the last decades. In the research field of anti-fouling and anti-coagulation of materials, this phenomenon is of particular interest due to its ability to prevent cell-adhesion in medical devices and implants. Even though publications including the new advanced fabrication methods of superhydrophobic materials are being widely spread, the role of the nanostructured surface remains unclear. Reports reveal contradictive results in the stability of the superhydrophobic state, and the influence on all interfacial factors involved in cell response remain unsure. Elucidating and understanding the underlying technical and physiological mechanisms involved in the utility of superhydrophobic materials is obligate to ensure optimal biocompatibility. To establish an accelerated movement from superhydrophobic fabrication method to medical applications, the reasons behind the conflicting results and theories should be revealed. Only then superhydrophobic surfaces can be transformed from theory to practice.

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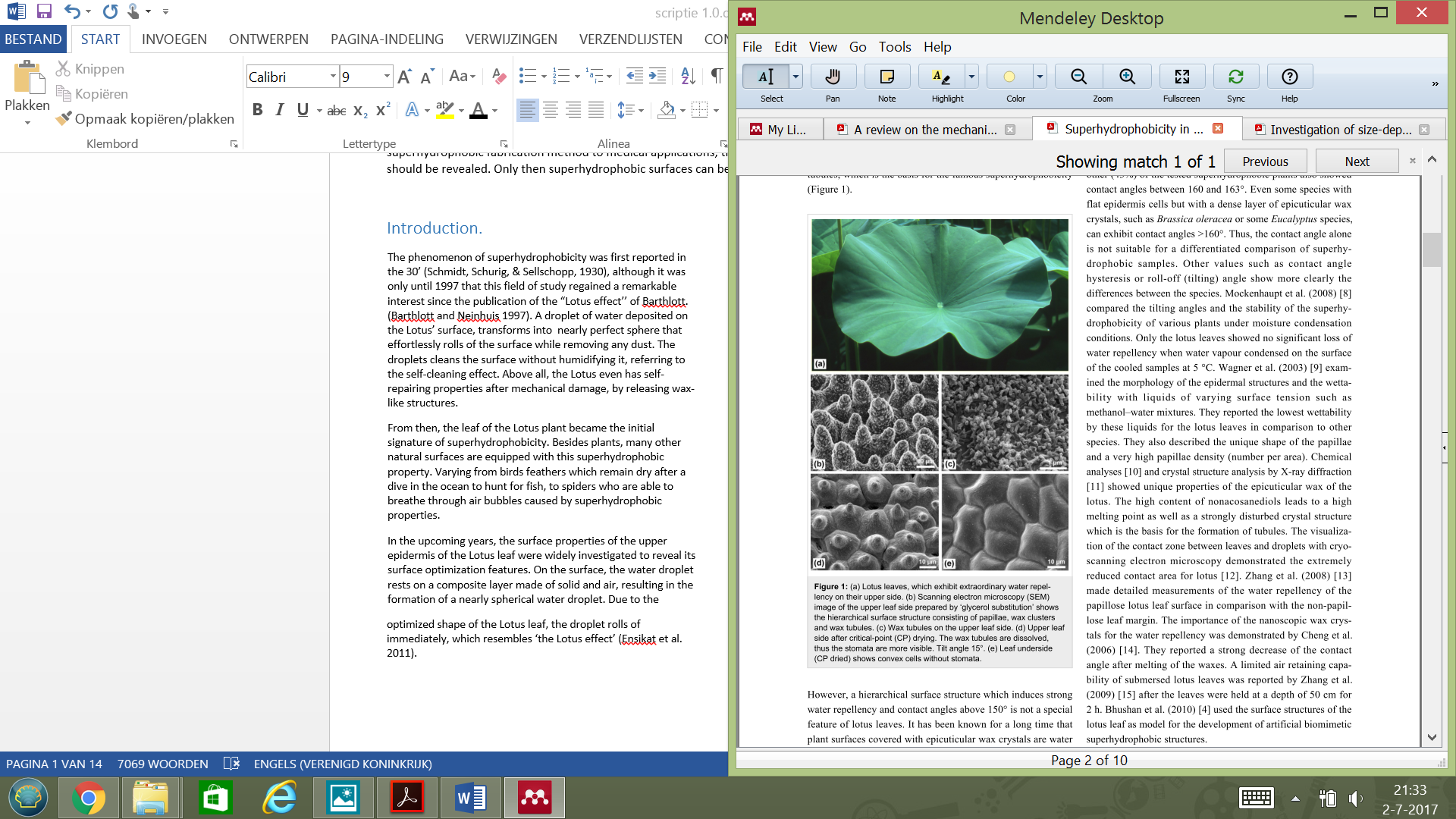
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Introduction.

The phenomenon of superhydrophobicity was first reported in the 30’ (Schmidt, Schurig, & Sellschopp, 1930), although it was only until 1997 that this field of study regained a remarkable interest since the publication of the “Lotus effect’’ of Barthlott. (Barthlott and Neinhuis 1997). A droplet of water deposited on the Lotus’ surface, transforms into nearly perfect sphere that effortlessly rolls of the surface while removing any dust. The droplets clean the surface without humidifying it, referring to the self-cleaning effect *(Figure 1)*. Above all, the Lotus even has self-repairing properties after mechanical damage, by releasing wax-like structures. From then, the leaf of the Lotus plant became the initial signature of superhydrophobicity. Besides plants, many other natural surfaces are equipped with this superhydrophobic property. Varying from birds’ feathers which remain dry after a dive in the ocean to hunt for fish, to spiders who are able to breathe through air bubbles caused by superhydrophobic properties. Subsequently the surface properties of the upper epidermis of the Lotus leaf were widely investigated to reveal its surface optimization features. On the surface, the water droplet rests on a composite layer made of solid and air, resulting in the formation of a nearly spherical water droplet. Due to the optimized shape of the Lotus leaf, the droplet rolls of immediately, which resembles ‘the Lotus effect’. (Ensikat et al. 2011)

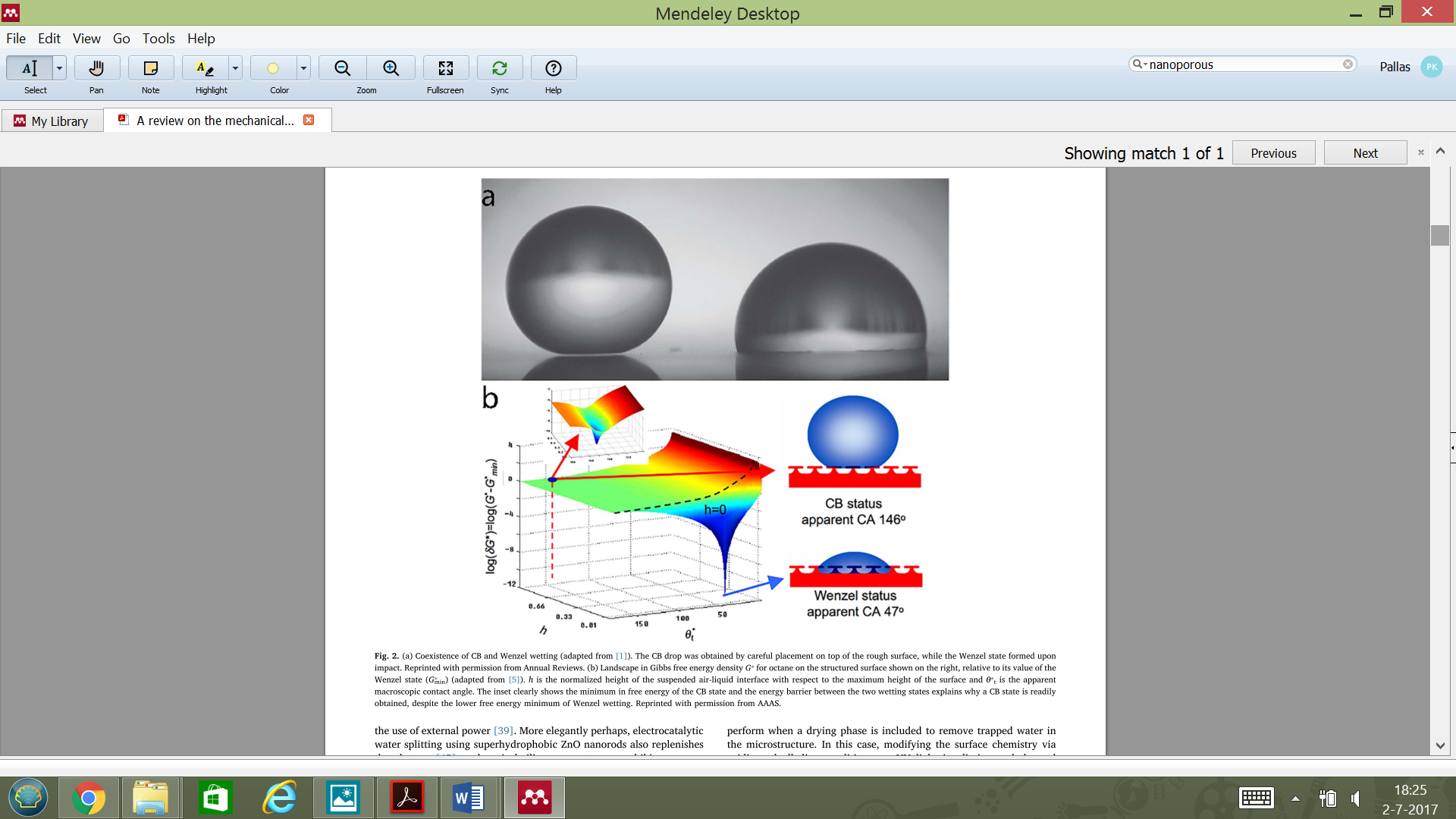
In the last decade, the advancements and utility of superhydrophobic materials have been significant. The potential of superhydrophobic materials are of interest for various fields of study and industries.

In particular in the field of tissue engineering and Regenerative Medicine, the superhydrophobic surfaces have the favourable feature of preventing the adhesion of proteins and cells, such as bacteria. Superhydrophobic coatings belongs in the type of anti-adhesive coatings. An advantage of superhydrophobic coating is their ability to reduce biofilm formation under fluctuating shear, depending on the strain involved. This property is of particular interest for implanted prostheses, or cochlear implants for example. A superhydrophobic surface is said to prevent the adhesion of bacteria as they need a transport medium, such as water to initially bound to a surface. From the moment bacteria adhere to a biomaterial, they surround themselves in an extracellular polymeric substances matrix (EPS), this matrix provides shelter against the host immune system, detachment forces and even against high levels of antibiotics. (Wu et al. 2014) Biofilms on biomaterials implant and devices and biomedical equipment could result in a high morbidity or even mortality to the implant host. This would lead to a noteworthy decrease in the failure of medical implants and therefore the operation costs. However, the use of superhydrophobic surfaces for biological of industrial application seem to inhibit by contradictive results of the cell-adhesion behaviour and stability. Even more, superhydrophobic coating also have the disadvantage of not enabling tissue integration. As the population is ageing quickly, the demand for medical devices has been rapidly increased. Understanding the relationship between the external factors involved in cellular responses and protein adsorption to superhydrophobic materials are crucial to determine their biocompatibility in the tissue-specific region. For example, for biomaterials implanted to interact with blood, such as in blood vessels or with artificial hearts, it is necessary for the biomaterial to not induce plasma protein adhesion to prevent thrombosis. (Hsu et al. 2013) To elucidate the challenges in cell-adhesive behaviour on superhydrophobic surfaces, publications should be enquired thoroughly. Detailed understanding of surface interfaces and adherence behaviour will provide the necessary information for a rational design of implantable medical devices or medical tools. (Thevenot, Hu, & Tang, 2008) This is fundamental in order to establish an accelerated use of superhydrophobic in real-life tissue-engineering and regenerative medicinal applications. In this review, the superhydrophobic effect is explained by the Cassie-Baxter and Wenzel wetting states. Besides, adhesive factors involved in the integrity of superhydrophobic properties and cell adhesion behaviour are discussed. In the end, suggestions are made to uniform the testing methods of superhydrophobic properties, and to develop protocols, as these are the key sources for a better clarification on cell-adhesive behaviour on superhydrophobic properties.

*Fig. 1 a) the Lotus leaf. b) Scanning Electron Microscopy shows surface structure. c) Wax tubules on leaf surface.*

Water Contact Angle.

To define wettability, the Water Contact Angle (CA) *(Figure 2a)* of a surface is the main used method for characterization. The CA is a measure of the surface tensions the between the solid-vapour solid-liquid or liquid-vapour interfaces (Young 1805). In general, if the CA is 0 ≤ θ < 90, the surface is termed hydrophilic, whereas if the CA 90 ≤ θ < 150 the surface is termed hydrophobic. Furthermore, superhydrophobic surfaces are commonly defined as a surface with a CA of > 150°. Although, strictly following this terminology, a surface with a water contact angle of 89 ° is called ‘’water loving’’, but changes into ‘’’water fearing’’ with only 2° increase to 91° water contact angle. Even though this is a widely used and recognizable definition, the boundary seems not to be strongly supported with technical data. Is this strict boundary proven to have a molecular origin? And how does a change of only 2° affect the expected cell-adhesive behaviour? The CA is well-known to be affected by the surface chemistry and surface geometry (Bracco & Holst, 2013). Commonly, the CA is measured with water in a liquid phase, both statically and dynamically. In the statically measurement, a droplet is placed on the solid composite surface, and measurement without any further manipulation. Where as in the dynamically CA measurement, the droplet size and volume is being modified. The measurement of the CA is favourable, as it is inexpensive, rapid and easy to learn. With goniometers, cameras and drop shape analyses the CA is most commonly measured (Kwok & Neumann, 1999). Even though the measurement of the water contact angle is easy to use, mistakes and inaccuracies still remain present (Kwok et al., 1998). Using the water contact angle alone, like some publications do, have arisen a lot of discussions, other reports explain the limitations of the current water contact angle measurements.

There is no widespread agreement on the droplet volume used to assess the water contact angle. Even the influence of gravity can be hazard in the perception of superhydrophobicity. The Novel Gravity model indicates that the change in volume, results in a change of internal hydrostatic pressure and therefore affects the droplet morphology. (Hu, Liu, & Zhang, ) Hence, that this droplet deformation is caused by the volume of the droplet and its gravitational force rather than reflection the real surface wetting situation. This could manipulate the judgement of superhydrophobicity, and when used as a biomaterial will result in unexpected and probably unwanted cell-adherence behaviour.

*Fig. 2. A: Respectively the Cassie Baxter state and the Wenzel State of a water droplet. The Cassie Baxter state was obtained by a placement with little force, while the Wenzel state was formed by force with impact. B: Graph showing the Gibbs free energy per state. H stands for the ratio of height of the air-liquid interface with respect to the maximum height of the surface. Θ stands for the contact angle. This schematic shows the energetic barrier between the Cassie Baxter state (red) and the Wenzel state (blue).*

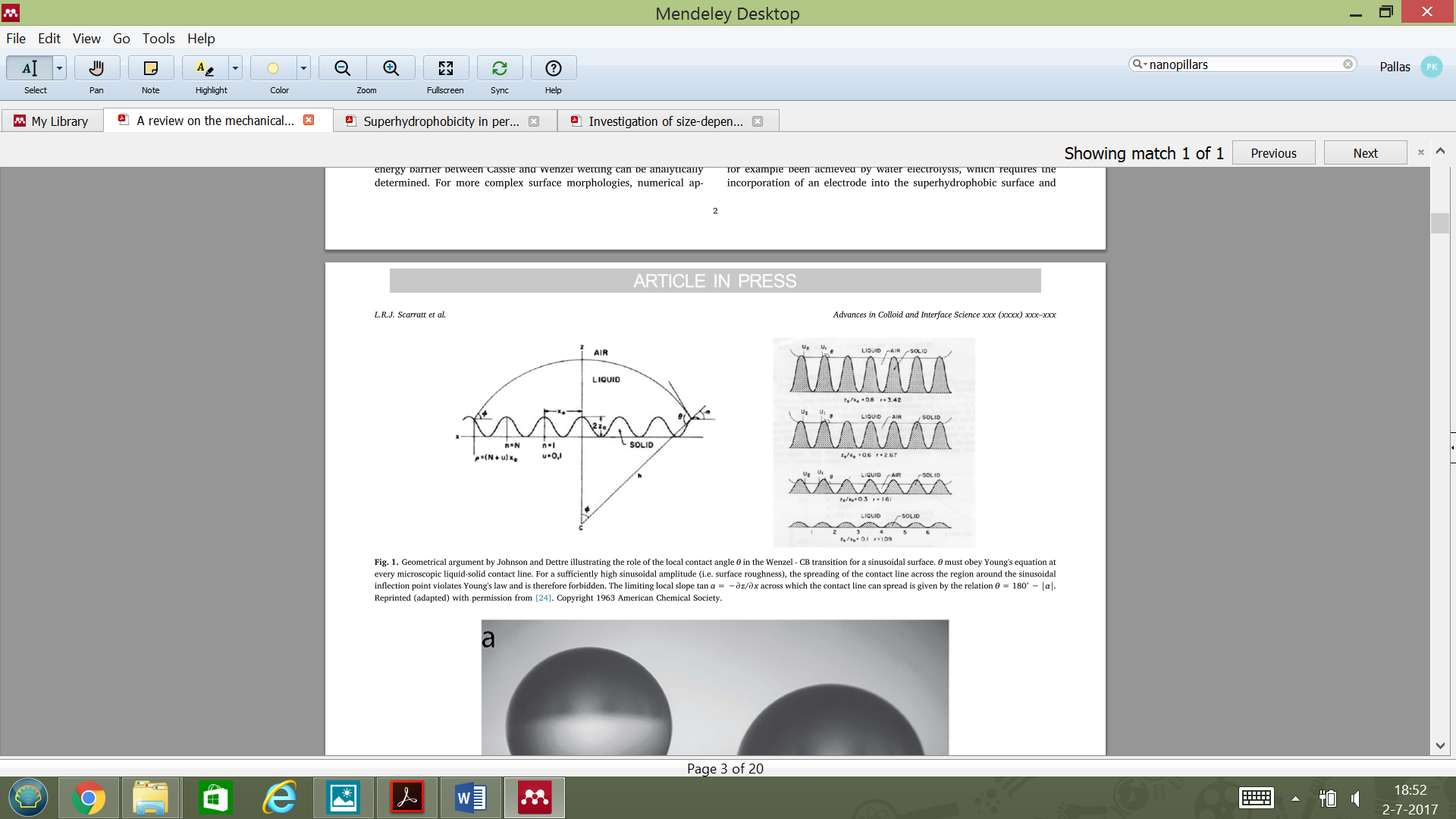
(Scarratt et al., 2017)

Cassie-Baxter State.

To mimic superhydrophobic surfaces inspired by nature, such as the Lotus leaf or gecko feet hairs (Autumn and Gravish 2008), researchers have developed various methods. The superhydrophobic effect cannot be achieved by simply choosing the correct material. To acquire this property, the material needs an ordinary low-surface energy, and therefore needs surface modification. The importance of the nanoscale roughness has been widely reported (Kuo, Chueh, & Chen, 2014). All methods are focused on obtaining a favourable surface roughness, inspired by the Cassie-Baxter and Wenzel models. In 1936 Wenzel suggested that the effective surface area will increase as the roughness of the surface increases. Therefore, water will spread more on a rough hydrophilic substrate to increase the solid-liquid contact, and spread less on a rough hydrophobic substrate to decrease the solid-liquid contact.

The CB state is also seen on the lotus leaf, where an increased surface roughness results in the trapping of air (termed a plastron) between the solid composite and the water droplets *(Figure 3).* This state is then thermodynamically favoured, as the system would be in a higher energy state when all of the hydrophobic substrate would be in contact with water, termed the Wenzel state *(Figure 2b).* This effect is essential for the understanding of cell-adherence behaviour on superhydrophobic surfaces. The modification methods to obtain this surface property can be grouped in ‘top down’ or a ‘bottom up’ approach. For the top down approach, the surface is modified through the removing of material to increase the surface roughness. Several methods have been developed by use of external power such as, templating, lithography, thermal heating, UV light irradiation, electrochemical etching or plasma treatment (Scarratt, Steiner, & Neto, 2017). The widely most notable top-down approach to compose superhydrophobic surfaces is lithography. Several versions of lithography have been developed over the years and are known for their easy and pinpoint control of topography properties and morphology. The modification principal of all versions is similar; exposure of electromagnetic radiation, which results in chemical changes in the surface molecular structure. (Acikgoz, Hempenius, Huskens, & Vancso, 2011; Chen, Gong, Li, & Li, 2016)

Although many reports have used modified surfaces to analyse cell adherence behaviour, the discussion remains if the non-adhesive cell-response is due to surface wettability or other mechanical surface properties. For the prediction of cell responses, the quality and durability of the wetting state is essential. To determine a critical condition for the transition between the two states, a handful of models have been published. These models include the bending of the micro menisci, dynamic mechanism, the liquid-gas interfacial energies (Reyssat, Yeomans, & Quéré, 2008), and the pin-effect of the surface texture (Moulinet & Bartolo, 2007). But the models have not been able to fully prove their accuracy, as their micro/-nano structures are hidden at the liquid and solid interface.

**To provide the necessary spatial resolution to optimize the analysation of the micro/-nanostructures, an Atomic Force Microscopy (AFM) can be used. Although before using the AFM, an interpretation of the surface forces is necessary. Plus, the tip-sample interaction influences the surface and therefore the results. Hence, the AFM can give a close examination, but measurement imperfects remain an issue. (Journet et al. 2005) In practice, it is frequently unclear if the Cassie-Baxter or Wenzel wetting state is favoured, therefore the boundary conditions between the two states still remain an actively studied field. Hence, the idea that the observed Cassie-Baxter effect will always result in the inhibition of cell adhesion, is to be questioned. Reports show different cell-adhesion behaviour of surfaces thought to be superhydrophobic. The Cassie-Baxter and Wenzel models can be used to give surface wettability predictions. However, the use of these models quite often seems to be inaccurate. The conclusion whether the superhydrophobic Cassie-Baxter or Wenzel wetting state is favoured on a surface, should also include further external parameters such as; mechanical motion or pressure. Therefore, other researchers choose to further limit the included parameters by the measurement of the contact hysteresis (CH) or the sliding angle (SA) to determine the surface wettability.

*Fig. 3. Schematic of an increased surface topography with higher amplitude but equal wavelength.*

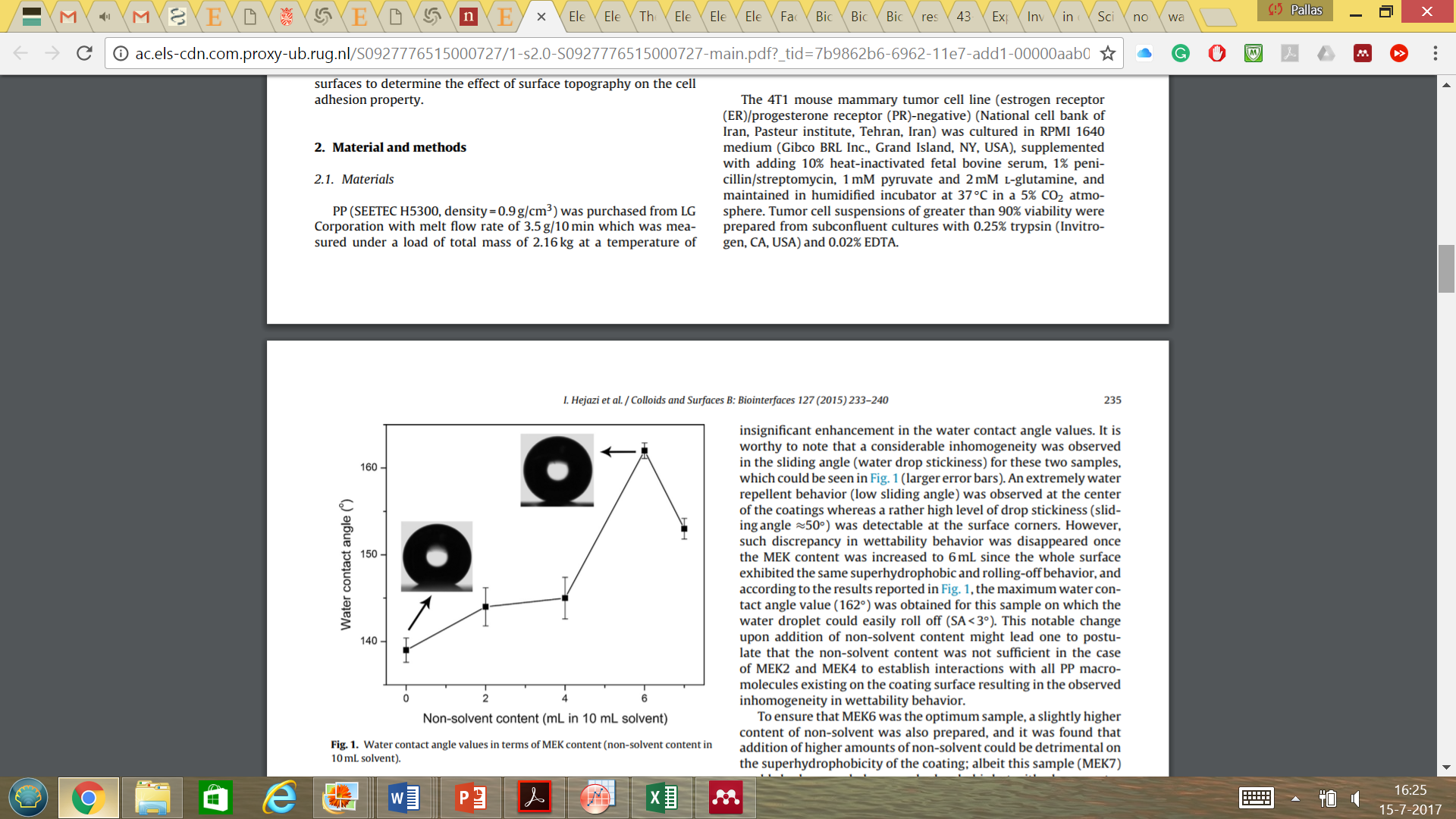
The CH is defined as the difference between the advancing angle and the receding angle and is generally dynamically measured by supplying and withdrawing volume to the drop. Low hysteresis defines the difference between the minimal and maximum local CA. A low CH on a superhydrophobic surface is favourable, as this means the water droplet can easily roll off the surface, removing any dust, termed as ‘self-cleaning’. The sliding angle depends on the mass of the droplet, and therefore can be used to compare different solid surfaces when a uniform droplet size is used. It is claimed to define a solid surface superhydrophobic if the static water CA is > 150 degrees and the CH is < 10 degrees. Several reports are known using improved models to give insight in expected outcomes.

The question arises if these plastrons alone are responsible for the cell-adhesion behaviour, or if other parameters have a stronger influence on the stability of the state than previously thought. The possibilities of other environmental influences should be taking into account for a qualitative assessment of superhydrophobicity and its potential applications in medical devices.

Nanopillars

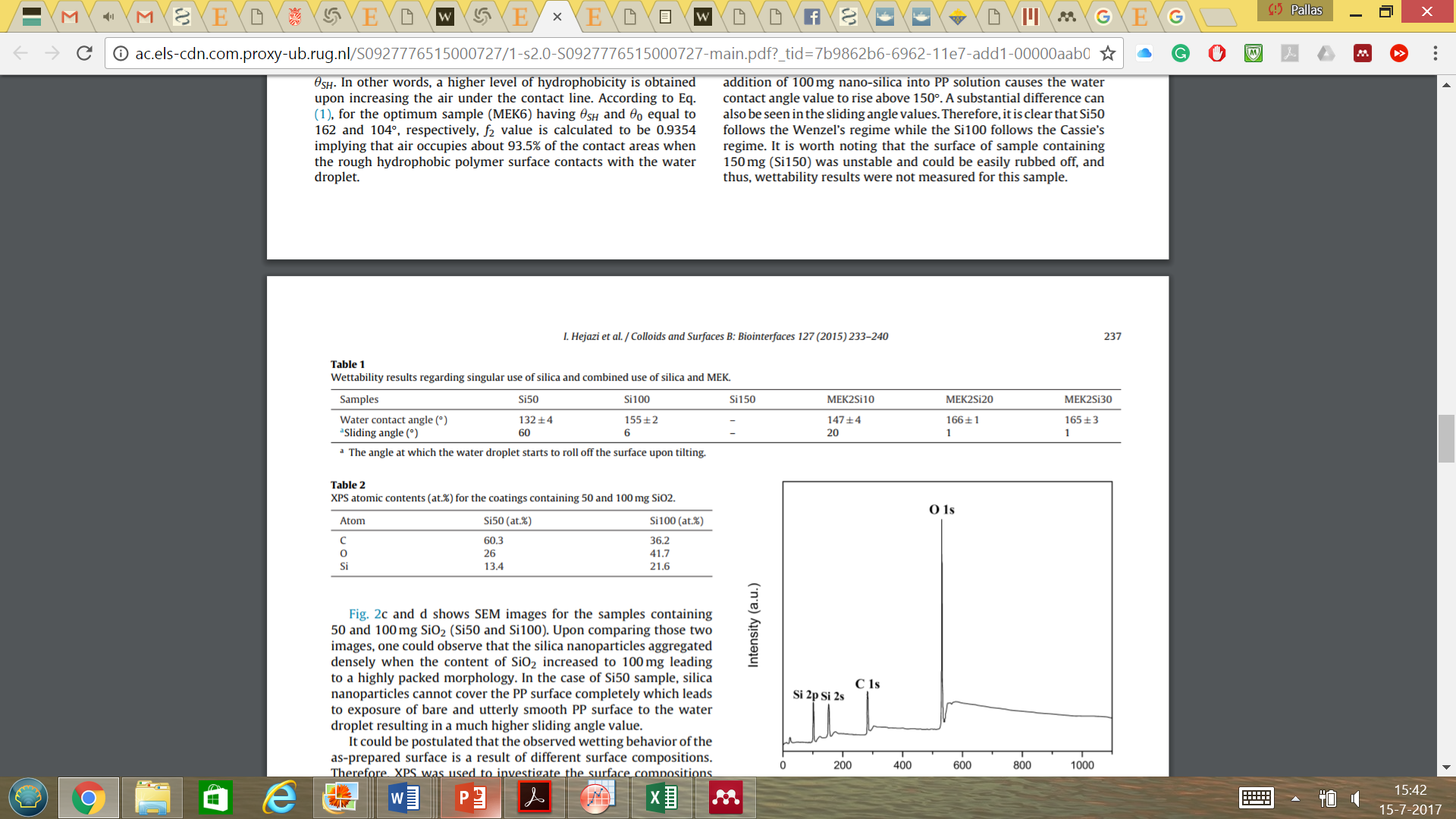
Various methods have also been developed for the bottom-up approach in the synthesis of micro-nano-array surfaces. In an attempt to mimic the topography of the gecko’s adhesive foot-hair, Jin et al. (Bang Lee, Jin Jeong, & Ro Lee) developed a fabrication method using polystyrene nano-tubes. Subsequently, other methods were developed using carbon nanotube (ACNT’s) arrays to fabricate honeycomb and rice-like structures. The fabrication of nanopillared surfaces is the most recent and advanced method in the field of superhydrophobic to generate surface roughness. Nanopillars are nanostructures with an approximate diameter of 10 nm and have a pillar shape on the bottom with a pointy top. These structures are also to be found in nature on the wings of a Cicada (Watson and Watson 2004). Mimicking these pillars is normally obtained by lithography treatment, and are popular due to its easy control of dimensions.

Hejazi et al. suggests that on these nanopillared surfaces, cells will not be able to reach the bottom of the solid composite, but will be floating on top of the nanopillared tops having local contact areas depending on the size and shape of the nanopillars and cell type. His report revealed that the apoptosis rate increased as the nanopillar size decreased. These results showed that the size of focal adhesions formed on these nanopillars decreased as the size of the nanopillars decreased. When the diameter of the nanopillar was decreased to smaller than 200nm, the shape of the focal adhesion points showed to be cell type dependent.

The theory that the pillar size is the original reason behind the non-adhesive cells response is yet to be discussed. Following the suggested mind set, the report of Hejazi et al. shows contradictive results. Why does the apoptosis rate increase when the nanopillar sizes decreased? If the nanopillar size decreases, the floating of cells would be made easier to be overcome, and cell adhesion would increase. Additionally, in this report, the water contact angle of non-structural sample MEK6 has a water contact angle of 165° *(Figure 5)* and the nanostructural MEK2Si10 and MEK2Si20 have a water contact angle of respectively 147° and 166° *(Table 1)*. Even though the water contact angles are all to be have a high wettability, the cell viability (%) is significantly different for MEK2Si10 and MEK2Si20 *(Figure 4)*. Where MEK6 has a cell viability of 40-55%, MEK2Si20 and MEK2Si20 have a decreased cell viability to even 5-10%. The argument that wettability in this case would ensure a different cell viability is therefore not sufficient enough.

*Fig. 4. Cell viability of pure polypropylene surface (PP), MEK6 and MEK2Si10 and MEK2Si20 after 3 and 5 days of culturing. Where Si10 and Si20 stands for the amount of nanosilica surface structure: 10% and 20 % of nanosilica with respect to MEK. MEK: Methyl Ethyl Ketone. Cells were 4T1 mouse mammary tumour cells.*

*Fig. 5. Water Contact Angle of MEK6 at 165°. Methyl Ethyl Ketone with 6 ml in 10 ml p-xylene solvent. At 6 ml, the water contact angle is ±166°.*



*Table.1. Water Contact Angle of MEK2Si10;20;30.*

**The mechanical damage of nanopillars.**

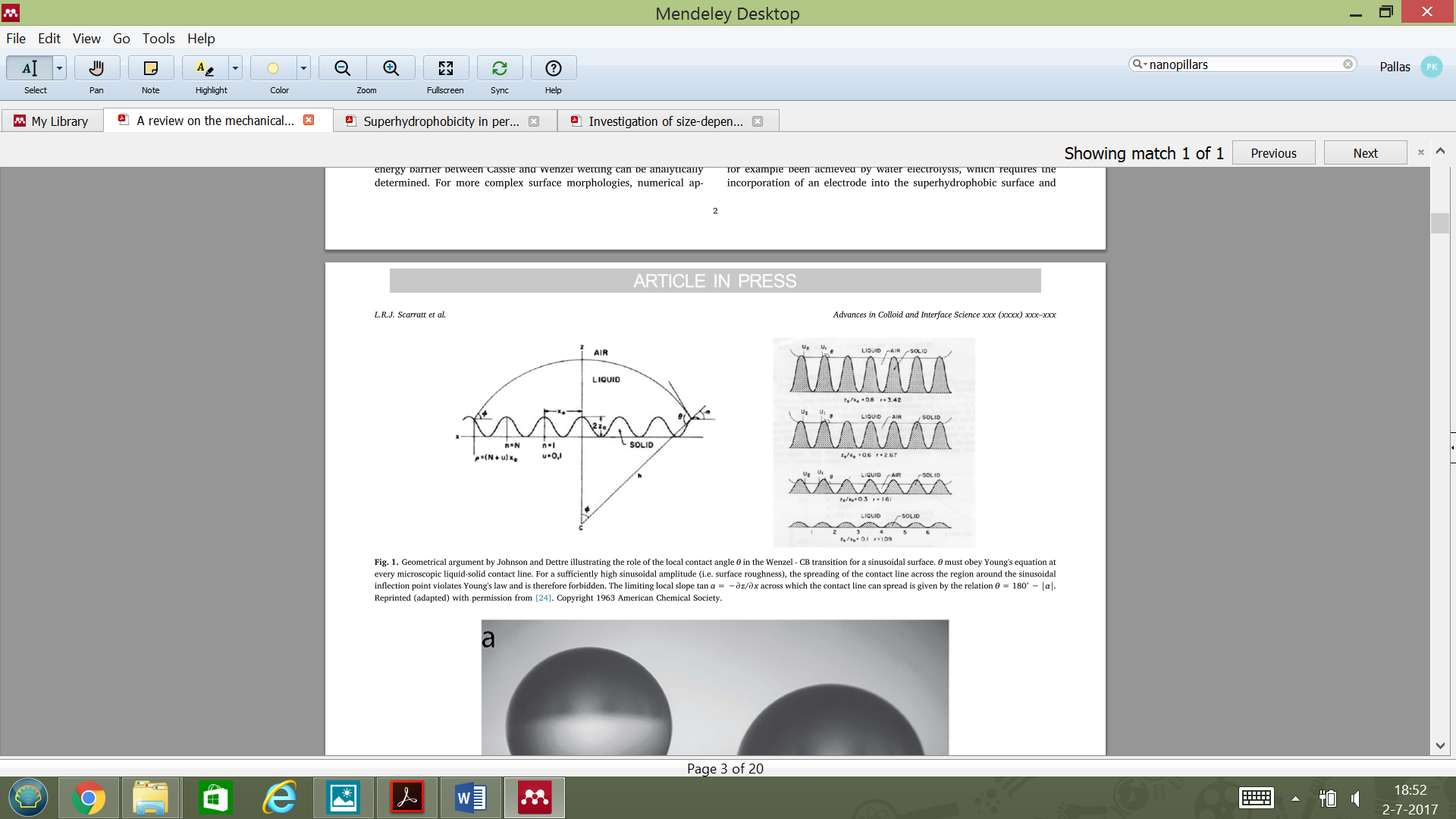
Potentially, this apoptosis is due to mechanical damage instead of biochemical interactions. Australian researchers reported the special nanopillar patterns of the Cicada *Psaltoda claripennis.* These researchers reported that the bacteria are pierced and killed by the pointiness of the surface. The nano spikes deforms and penetrates the bacteria, instead of chemical interactions. The killing prevents the bacteria from adhering. This so called ‘bactericidal activity’ on these type of surfaces is the result of mechanical damage, and helped designing antibacterial materials. In this case, the bacteria could be seen to be repelled by the surface, as little to no adhered cells are observed. Hence, in the report of Hejazi et al. the decreased cell viability is potentially resulted by bactericidal activity. Therefore, nanopillared surfaces should not be considered to be ‘’anti-fouling’’ before excluding bactericidal activity.

**Protein absorption and demolition of superhydrophobicity.**

Another interesting discussion referring to the report of Hejazi et. al; is there finding that the cell inhibition is significantly present if the surface nanostructure is small. MEK6 was measured to have a nanostructural diameter of 1-2 µm and a cell viability of 40-55%. Questionable is the origin behind this finding; Is there another indirect influence for the decreased cell viability? Other research shows that small nanostructured topography induces protein absorption. As proteins adhere to the surface, the local surface tension decreases. A small number of substrates or adhered proteins can change the surface energy and tension. If the surface tension decreases to a critical point, the plastron is not trapped with high stability. Therefore, the plastron can escape and the superhydrophobicity is partially or totally demolished. As a result, cells are not floating on top of the nanostructures, and are able to adhere to the substrate (Mohammadi, Wassink, and Amirfazli). As the report does not include protein absorption analysis, it is not to be excluded that protein absorption is the origin behind the cell inhibition.

Weighing factor of surface properties.

Besides the nanopillar diameter size, research shows significant different findings in the influence of topography amplitude size in cell adhesion and focal adhesion sites. Reports show that an increased nanostructural amplitude with a constant diameter, will result in a decreasing stability of the Cassie-Baxter state. This effect is partially proven to be true because of the local contact angle dependence of the Cassie-Baxter and Wenzel states. With a higher amplitude, the local contact angle changes, and will pass the critical angle of Cassie-Baxter preferable state. The contact angle increasing as the roughness amplitude of the surface increases. *(Fig. 6)* As earlier mentioned, the Cassie-Baxter state will not be stable and will transform into the Wenzel state. In the Wenzel state, all solid surface area will be in contact with water. (Yang, Tartaglino, and Persson 2006) Due to the complexity of data that can be analysed while experimenting with cell-response behaviour to different surface topography, reports remain to show contradictive results.



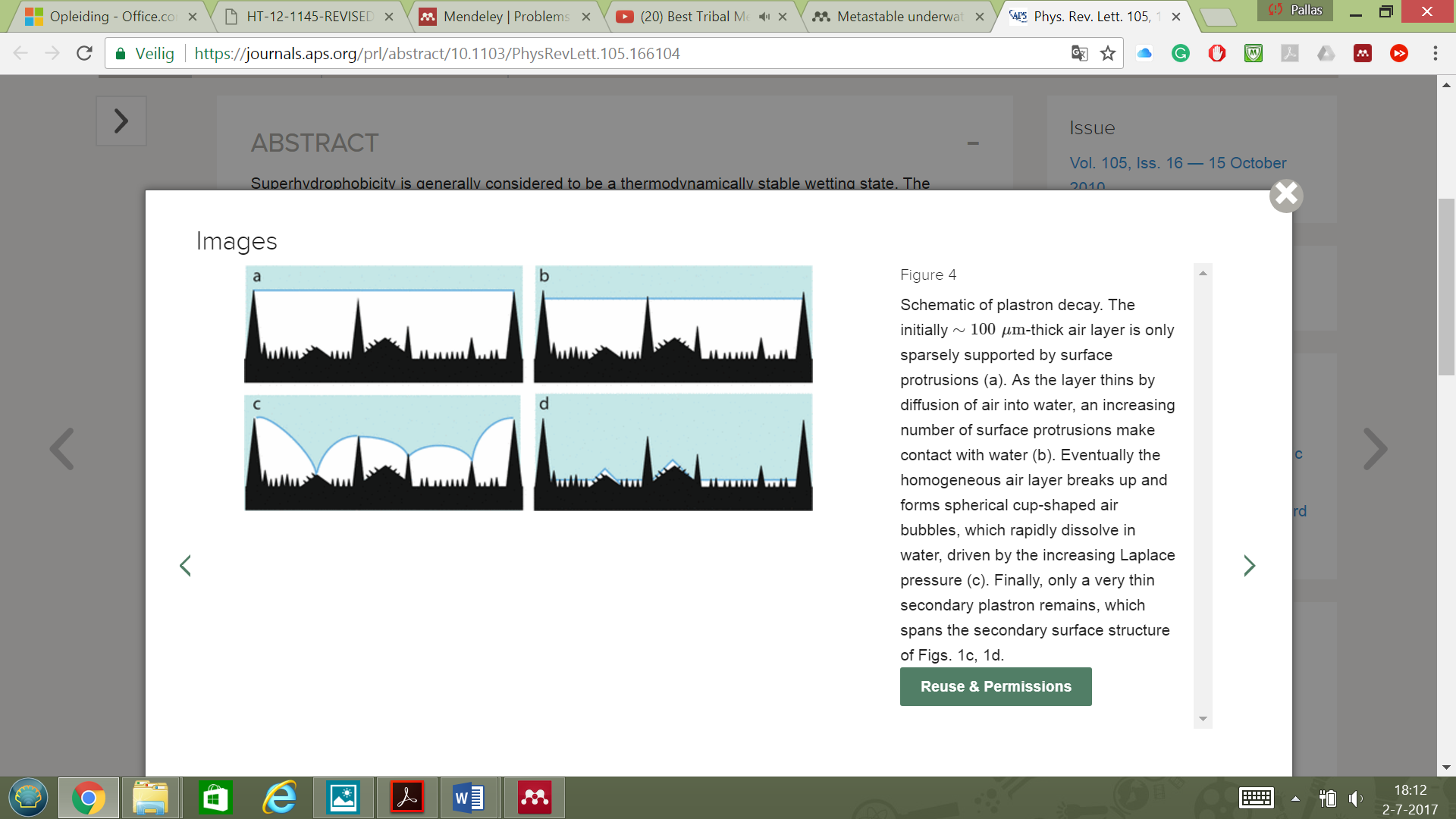
Surface roughness and chemistry are known as critical factors influencing the cell-behaviour on biomaterials. Although, little seems to be known about the weighting factor of both properties. What is their relative influence to the superhydrophobicity of the surface? Hallab et al. reported that the surface free energy of the substrate was of a higher importance than the surface roughness for the strength of cell adhesion and its further proliferation. On the other hand, Ponsonnet et al. describes experiments on titanium and titanium alloys where the surface energy was a superior factor for adhesion and proliferation, but that with a combined surface roughness, the cell adhesion and proliferation is interrupted. A roughness threshold seems to be between (0.08-1 µm). The specific influence of all surface properties should be further explored to elucidate their underlying mechanisms.

*Fig. 6. Schematic of the role of the local contact angle in the transformation between the CB and Wenzel state. The dimensions of the sinusoidal surface determine if the critical local contact angle is violated, which is giving by: related to*

*American Chemical Society 1963.*

Stability of the plastron.

For the application of superhydrophobic surfaces, the thermodynamic and kinetic stability are very important to ensure its water repellent properties. For the thermodynamic and kinetic stability, the resistance of the plastron to transit into a Wenzel wetting state is a major factor. If the energetic barrier decreases to a critical point, gas escapes out of the roughness, and the surface features will be filled with water and loses its water repellence, changing into the Wenzel state. By local condensation, high pressure, droplet evaporation or hydrostatic pressure, the water droplet could transform in the vapour phase and irreversibly transforms into the Wenzel wetting state *(Figure 7).* The reverse process of the formation of a plastron by air is thermodynamically more difficult to realise. Like previously mentioned, the local contact angle has been found to play a main factor in the determination of the state, and little is known about the influence of environmental change in parameters for the stability of the state (Scarratt et al., 2017). One environment that has been recently explored, is the emerging of rough surfaces into water. Even though superhydrophobic surfaces are found in nature at plants, birds or spiders, natural occurring underwater superhydrophobicity seems to be absent. (Poetes, Holtzmann, Franze, & Steiner, 2010) The stability of the plastron in the Cassie-Baxter state is miscible when the solid composite is submerged. This effect was firstly described the Herminghaus in 2000, when he submerged a lotus leaf at a depth of 20 cm’s for a few second, and observed the changing into a Wenzel state (Herminghaus, Brinkmann, and Seemann 2008). This phenomenon has also been reported for Teflon surfaces with a hierarchical roughness. The decay of the plastron has been described in two phases, starting with the thinning of the plastron, followed by the transformation of the plastron into surface bubbles.(Yao et al. 2013). Taking in to account the increasing amount of publications reviewing superhydrophobic effect, the number of reports investigating the underwater stability of the plastron is surprisingly rare. This seems to be curious, as this effect should arise many interesting questions.

Current nanotechnology methods have allowed researchers to model the flow in nano/- micro channels better than ever before. Methods for measuring the plastron thermodynamic robustness usually include applying pressure on the plastron for stability tests, immersion tests and compression tests. A droplet bouncing and impact tests can be used to investigate the thermodynamic robustness of the plastron. To evaluate the topographical damage and durability of the surface, the mechanical robustness of the plastron in the surface roughness can also be evaluated by the use of an abrasion tests. Abrasion tests are used to emulate environmental damage done at the micro-nano topography. To mimic environmental shear forces, like sand abrasion from wind, silica particles are dropped from heights and let vibrating or by letting the particles vibrate on the surface. Recently, abrasion tests including water jet tests examined the thermodynamic robustness of the plastron and its mechanical resistance to applied shear forces by water. Additionally, a Cavitation tests can be assessed to evaluate the surface robustness against water. By applying sonication for an extended time, the topography damage can be explored due to the two-phase decay of the plastrons. When the plastrons explode, they release an enormous amount of energy and high local shear. By tracking the surface wettability at different time intervals, the topography damage due to this effect can be exposed. (Scarratt, Steiner, and Neto 2017) (Emelyanenko et al. 2015)

*Fig. 7) Plastron decay in a schematic overview. A) The plastron is trapped with little contact points. B) By diffusion of air into water, the plastron air layer thins which creates more contact points with water. C) The plastron thins out, forming spherical shaped bubbles. D) As the spherical shaped bubbles are thermodynamically unstable, these quickly dissolve in the water by the increasing hydrostatic pressure. This results in the last state, with increased surface-water contact area.*

*Poetes et al. ‘’Metastable underwater superhydrophobicity’’*

The simulation methods give revolutionary information, but the molecular flow dynamics in hydrophobic, hydrophilic and emerged nano-channels still remains difficult to be implemented. (Sofos et al. 2016). Research has entangled pieces of the influence of pressure, flow speed and tensile strength on superhydrophobic surfaces for other commercial application. Nevertheless, these results are not an accurate and precise model for a in vivo tissue specific environmental parameter. Hence, for an accurate prediction of the wetting state, more researching in the underwater stability of superhydrophobic is necessary (Falde et al. 2016).

An in vivo research of the stability of the plastron is hard to realize, due to many experimental challenges. The lack in in vivo experimental data makes it inevitable to understand short and long-term cell behaviour to superhydrophobic surfaces. This makes it difficult to prevent failure of superhydrophobic properties in medical devices, and are therefore mostly investigated in the occurrence of implant/- or medical device failure.

Bacterial adhesion.

A fundamental problem using superhydrophobic materials for non-adhesive materials is that it is difficult to conclude a surface non-adhesive for all cell types. Experiments are usually done with one cell type, giving a type specific result. Every cell type has different responses, like so between mammalian cell and bacteria there is big physicochemical variations, with unequal surface responses. One characterised difference is that mammalian cells, like osteoblasts are commonly around 10-100 µm in diameter, and are known to have flexible membranes which can adapt to the environmental substrate. On the other hand, bacteria are only a few micrometres in size and are distinct in shape. Staphylococci bacteria, which are often associated with biomaterial infection,

Among these studies, some researchers found an increase of bacterial attachment to surfaces features smaller than 100 nm (Park, Banks, Applegate, & Webster, 2008), while others reported a repellent effect to nanostructural surfaces even though the spatial distance was alike. (Díaz, Schilardi, dos Santos Claro, Salvarezza, & Fernández Lorenzo de Mele, 2009). The bacteria *Pseudomonas fluorescence* used by both reports has a size of 1500 to 5000 nm, although the space between the nanostructures in Diaz et al. was measured to be between 100-200 nm. If the dimensional topography of nanostructures is smaller than the size of the bacteria, bacteria are not able to attach to the solid composite as the bacteria floats on top of the structures. It seems to be due to the CB effect that the bacteria are not able to adhere, although sometimes it is just dependent on the bacterial dimensions.

Just like other cells, bacteria need contact area to release adhesion proteins to survive. Due to the modified roughness and topography, bacteria are dependent on the size of these surface features to be able to adhere. Bacteria are dependent on cell-cell communication to establish a firm adhesion, what is found to be decreased if the spatial roughness size is smaller than 250 nm. These findings should be taking into account when analysing bacterial adhesion behaviour.

Although superhydrophobic rough surfaces are thought to repel bacteria in theory, there are even some arguments to say rougher surfaces even induce bacterial adhesion. A rougher surface means more surface area for attachment, while protecting the bacteria from shear forces. Besides, hydrophobic biomaterials are known to have a high affinity for various proteins which induce electrostatic interactions with bacteria. Focusing on the influence of the surface topography on adherence behaviour, cells have been more widely investigated than bacteria.

Conclusion & Discussion

In addition to the roughness of surface nanostructures, the dimensions and physiology of all surface molecules, including density, surface contact area and spacing are found to be major physical factors for cell adhesion and spreading. Even more, a misunderstanding in the field of superhydrophobic interfaces, seems to be the idea that cells will have no chance to adhere, due to the Cassie-Baxter effect. Many reports prove that adherence of cells still is inevitable to some degree. Cells that adhere to the surface have been reported to change of shape into a more narrower formation, but still proliferate and have metabolic activity. Cells maximize their contact area between cell and surface by aligning themselves with the found nanostructure. Besides, this could also be a result of, accordingly to the Cassie and Baxter model, the not total contact of the cell with the fluids, like a medium suspension. (Hejazi et al., 2015)

As every cell type has their dimensions and characteristics, more research is needed to elucidate cell type specific surface response. Researchers should increase the parameters examined while analysis surfaces. For the understanding of cellular behaviour of various cell types and different materials, all components present on the surface should be explored, like cell shape, membrane rigidity, mobility and substrate sensing. Bacterial colonization is mostly noted as the average of the entire sample, without the analysis of the cell angle and position. Therefore, the relation between surface topography and microbial colonization is hard to establish. Furthermore, a big mistake is defining surfaces is to describe a surface by its fabrication method, instead of precisely defining surface topography measurements. The cell-response to a topography should therefore be considered to depend of the cell size and other surfactants. Next to superhydrophobicity, mechanical surface properties can be the reason for the non-adhesive cell response, like nanospikes membrane piercing. Even more, the topography dimensions could be too small for cells to sink to the substrate and are not able to adhere. In the future, it is needed to elucidate the underlying mechanisms responsible for these contradictive results. Besides, the weighting factor of these mechanisms when combined should be revealed. Although the water contact angle is widely used, it remains difficult to be measured accurately, due to numerous experimental challenges. Partially by to the lack of experimental evidence of fluid dynamics on a micro/- and nanoscale, different models lack in accuracy (Extrand). To exclude judgemental errors, a uniform method of measuring surface wettability is needed. Researchers should not only be focusing on the water contact angle itself, but should use more parameters to qualify and quantify the superhydrophobicity of a surface interface. Additionally, the stability of the plastron should be examined before the utilization of the biomaterial. The plastron is dependent of thermodynamically factors, which could demolish the superhydrophobic property. The stability of the Cassie-Baxter state should be critically reviewed under all conditions that matter for the potential medical application, to prevent the failure of the superhydrophobic properties. For an accelerated use of superhydrophobic surfaces in technological applications and establish a world-wide impact, researchers should move their focus on not only the development of new methods, but also obtain full characterization of surface robustness and durability. (Chen, Gong, Li, & Li, 2016) (Scarratt et al., 2017) Hence, world-wide protocols should be developed for more trustworthy and better comparable results. Besides, this enforces the discussion to not prematurely cite superhydrophobicity as reason for the nanostructured cell response. This will accelerate the understanding of factors involved in nanostructured surfaces, instead of giving contradictive results. In the future, this will accelerate the development of more biocompatible biomaterial surfaces and the movement of superhydrophobic surfaces into real-life medical applications.

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