White Paper

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BIOLIN SCIENTIFIC

Wettability Measurements in Biomedical Applications

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Wettability Measurements in Biomedical Applications

Wettability is of an utmost important property of a material that affects its response with the surrounding environment. In biomedical applications, wettability determines the material interaction with proteins, cells and bacteria. Wettability measurements are thus extensively utilized also in biomedical applications.

This white paper reviews some of the most used wettability measurement methods in biomedical device development. It also gives examples of how the measurements have been utilized in this research area.

An introduction

Surface wettability (terms hydrophobicity and hydrophilicity are often used) is one of the most important parameters affecting the biological response of an implanted material. Wettability affects protein adsorption, platelet adhesion/activation, blood coagulation, and cell and bacterial adhesion [1-2].

The selection of the material for biomedical applications is typically done based on its bulk properties. As the surface properties of the material are often not suitable in terms of biocompatibility, two different approaches have been taken; either the bulk materials are modified, or the coating is applied. Modification of the bulk material involves the incorporation of additives or the use of composite materials to increase biocompatibility. This approach has mainly been utilized in biodegradable material development [3]. Another option is to coat the material. Different types of coatings are commonly utilized in biomedical applications. Such examples are different polymeric materials used in and ex-vivo settings that are coated with hydrophilic coatings [4], and load-bearing metallic implants that need surface coatings to improve their interaction with the surrounding tissue [5].

In addition to surface properties of the coating itself, wettability is also important when biomedical coatings methods are developed. The success of biomedical coating depends on its proper and adequate adhesion on the target substrate, which is influence by the surface properties of the material [6].

Contact angle is a measure of wettability

Contact angle is a straightforward technique that can give quantified information on solid wettability. Contact angle measurement is easy to perform and an inexpensive method which makes it a preferred first-line characterization technique for biomaterials [7]. Contact angle can provide useful information on surface energies [8] and also give clues to the surface chemistry [9].

The contact angle is geometrically defined as the angle formed by a liquid at the three-phase boundary where a liquid, gas, and solid intersect. Three different forces are acting on this three-phase contact point between solid, fluid and fluid as shown in figure 1.

The γ_{lv} is the surface tension of a liquid, γ_{sl} is the interfacial tension between the solid and liquid and γ_{sv} is the surface tension of the solid i.e. surface free energy.

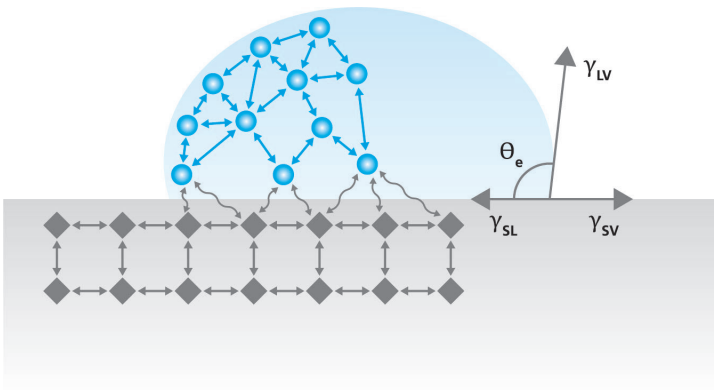

Figure 1
Three-phase contact point where solid, liquid, and gas meet.

The well-known Young´s equation describes the balance at the three-phase contact.

$\gamma_{sv} = \gamma_{sl} + \gamma_{lv}\cos\theta_Y$

The interfacial tensions, γ_{sv}, γ_{sl}, and γ_{lv}, form the equilibrium contact angle of wetting, many times referred to Young´s contact angle θ_Y.

Young's equation assumes that the surface is ideal. This means that it is flat, rigid, perfectly smooth, and chemically homogenous. Furthermore, it assumes that the system is stable i.e. there is no interaction between the liquid and the substrate.

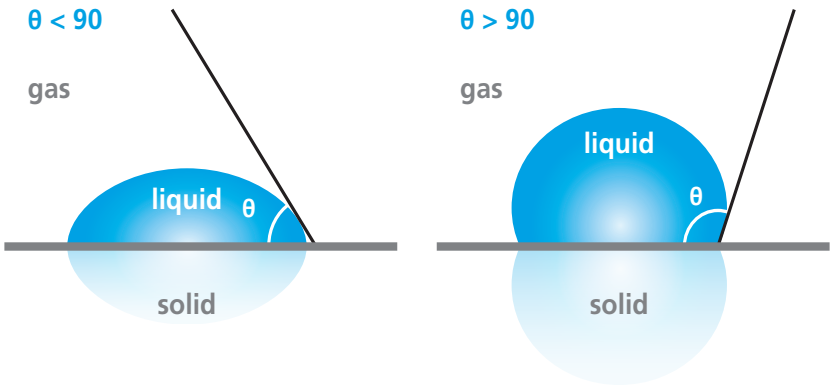

Figure 2
Drop of water on (left) hydrophilic and (right) hydrophobic surfaces

Sessile drop

Sessile drop measurement is the most commonly used contact angle measurement method. The measurement is done with an optical tensiometer which includes a high-resolution camera, a light source to enable droplet visualization, an automated or manual dispenser, and a manual or automated sample stage.

A drop of liquid is placed on the sample surface. The image of a drop is taken and the software gives the angle the droplet forms with the surface. The measurement can be fully automated to ensure repeatability.

Most typically water contact angles are measured. Based on that, the surface is classified either hydrophilic (contact angles below 90°) or hydrophobic (contact angle above 90 °) as shown in figure 2.

In biomedical research, hydrophobicity is used as an indicator of how the material will behave when in contact with the human tissue or body fluids. Protein adsorption is one of the first things that happens when the material comes in contact with the human body. It is a common understanding that proteins are more prone to adsorb on hydrophobic material. Protein adsorption in blood-contacting medical devices initiates a thrombosis cascade and bacterial infections. Protein adsorption also leads to biofouling which reduces the sensitivity of implanted biosensors and deteriorates the performance of permanent implants. Polymeric materials are often used in biomedical devices due to their bulk mechanical properties. However, their surface as such is typically not suitable due to their inherent hydrophobicity [10].

Measurement of surface wetting is also important when studying cell adhesion. In bone-contacting applications, the osseointegration mechanism starts when the implant gets in contact with the blood. To increase the implant surface area for human osteoblast adhesion, it is necessary to increase surface wettability [11].

Bacterial adhesion to polymeric substrates was found to be dependent on the static contact angle with moderate hydrophobicity of around 90 degrees showing the highest level of bacterial adhesion. Then on the other hand extremely hydrophilic and extremely hydrophobic surfaces reduce E.coli adhesion [12].

Sessile drop measurements are commonly utilized to determine the surface free energy of the substrate as discussed later in this white paper.

Another important aspect to consider is the surface roughness. Surface roughness will affect wettability and that should be taken into account when contact angles are measured. This is discussed next.

Sessile drop measurement can be used to determine the hydrophilicity or -phobicity of the surface.

Roughness corrected sessile drop

The measured static contact angle is not equal to Young's contact angle as the Young contact angle assumes the surface to be ideal. This is of course not the case with real samples that can be chemically heterogeneous and practically always have some roughness on it (see figure 3).

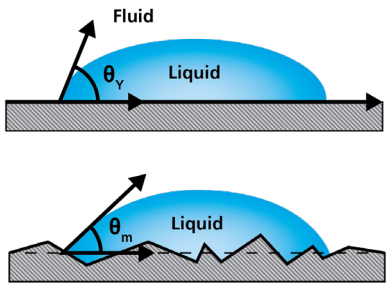

Figure 3
Droplet an a smooth (top) and rough surface (bottom). The measured contact angle is not the real contact angle on a rough surface.

The relationship between roughness and wettability was defined by Wenzel who stated that adding surface roughness will enhance the wettability caused by the chemistry of the surface [13]. Roughness corrected contact angles can be calculated by

$$\cos\theta_m = r\cos\theta_Y$$

where θ_m is the measured contact angle, θ_Y is roughness corrected (i.e. Young's) contact angle and r is the roughness ratio.

Measuring surface roughness together with contact angle makes it possible to separate the influence of surface chemistry from surface roughness on wetting and adhesion behavior. This is especially important when working with different types of surface modifications where both surface chemistry and surface topography are altered. To read more about the measurement and the theory behind the roughness correction, the reader is referred elsewhere [14].

In biomedical applications, it has been studied how the chemical and mechanical surface treatment affects the wettability, surface roughness, and surface energy of ceramic materials used in clinical dentistry [15].

Surface roughness as such is important in biomedical applications. It has been shown that surface roughness as small as 30 nm promotes bacterial adhesion on some metallic surfaces [16]. Micron and submicron scale roughness has also been shown to affect cell proliferation and differentiation on the material surface [17].

Picoliter sized drops

In some special cases, the area where the contact angle needs to be measured is extremely small. Picoliter sized drops can then be used. In biomedical applications, this applies to catheters, and other devices where the placement of a regular microliter sized drop is difficult or impossible [18]. An image of a picoliter sized drop on a sample is shown in figure 4.

In practice, the optical tensiometer is

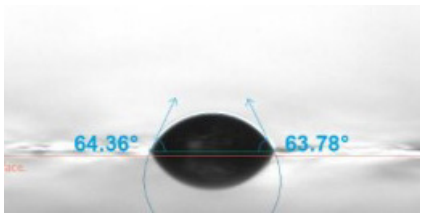

Figure 4
Picoliter sized water drop

equipped with a picoliter dispenser based on the piezoelectric actuation that shoots the drop on the surface. A high magnification lens is needed to visualize the drop on a surface. A high frame rate of the camera is typically also used, as the combination of small drop size, evaporation, and possible adsorption will make the drop lifetime short.

Care needs to be taken if measurements with micro and picoliter sized drops need to be compared as on some substrates the size of the drop can influence the results [19].

Captive bubble

A captive bubble is a method where the contact angle is measured in a reversed manner compared to the standard sessile drop (figure 5). The sample is placed upside down in a cuvette filled with water with the help of a specific holder. An air bubble is brought on the sample surface with a hooked needle and the contact angle of an air bubble is measured. The water contact angle of the sample is then reported as 180° minus the measured contact angle. The method can also be used if the contact angle measurements need to be done on a water immersed sample with some lighter liquid, such as oil.

In the biomedical field, the captive bubble is mainly utilized to study the wettability of a contact lens, and other hydrogel and wet substrates. The method has two benefits. First, the dehydration of the surface is prevented as the substrate is submerged into solution throughout the whole measurement. Second, water contact angle measurements on highly hydrophilic surfaces are generally difficult as the water will spread on the surface. Using the captive bubble will circumvent the issue. Note that in addition to the static contact angle measured with the captive bubble method, dynamic contact angles are also measured with the needle method, later described in this white paper. To read more about contact lens wettability, the reader is referred elsewhere [20].

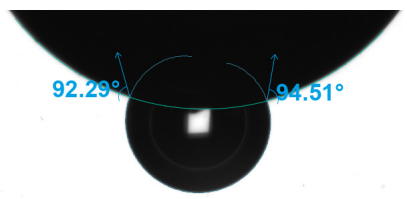

Figure 5
Captive bubble on a surface of a contact lens

Surface free energy can provide more information on the surface. Roughness correction enables the separation of surface chemistry and roughness.

Surface free energy for surface chemistry

The water contact angle can be used to categorize materials based on their hydrophilic/-phobic properties. Surface free energy gives a solid surface value similar to the surface tension of a liquid [21]. The contact angle is always dependent on the liquid used as well as properties of the surface but surface free energy is the inherent property of the solid.

Surface free energy is typically divided into polar and dispersive parts and in some cases, the polar part is further divided into acid and base components. Surface free energy cannot be directly measured but it is calculated through contact angle measurements. The more detailed description of the surface free energy theories and how surface free energy is calculated can be found elsewhere [22].

In biomedical research, the most used SFE energy theory divides the surface free energy into three different components; dispersive, acid (electron acceptor), and base (electron donor). One reason for this is that many organic polymers, especially many polar biopolymers are mainly only electron-donors, or to a lesser extent only electron-acceptors. The surfaces with only either electron-donor or electron acceptor properties are called monopolar. Such monopolar surfaces have unexpected properties that may give explanations to some well-known but poorly understood colloid and surface phenomena [23]. Also, the acid-base theory has been shown to give the most consistent results when evaluating the surface free energy of microbial cells [24].

Surface free energies are also correlated with bacterial adhesion. In the study of bacterial adhesion on various self-assembly monolayer coated glass slides with controlled terminating groups, it was concluded that higher the total and polar component of the surface free energy, less bacterial adhesion occurs. This is in good correlation with several other studies where the surface hydrophilicity has been determined to diminish bacterial adhesion. Moreover, the authors were able to correlate the bacterial adhesion to be mainly affected by the electron donor character of the substrate surface. The increase of the electron donor part (Lewis base) of the surface free energy decreased the bacterial adhesion [25].

In another study, the surface free energies are measured on various samples and correlated with fibroblast adhesion. It was found that the higher the surface energy, the higher the cellular adhesion. The strongest correlation was found between the total surface free energy and cellular adhesion strength, followed by correlation with the polar component. Less correlation was observed with the dispersive component [26].

As with static contact angle measurements, the roughness plays an important role also in surface free energy determination. This is discussed next.

Roughness corrected surface free energy

The surface free energy of a material, and what cells encounter, for example, is ultimately the same independent if the material is rough or not. However, as the surface free energy is determined by contact angle data on which the surface roughness can have a huge effect, it is of utmost importance to take the roughness into account. This can be done by using roughness corrected contact angles to calculate the roughness corrected surface free energy of your material.

It is typical to measure surface free energy and surface roughness as separate property when in reality they are bound together when surface free energy is determined through contact angle measurements. When roughness corrected surface free energy values are determined, it is easier to evaluate the effect of different properties of the surface to protein or cell adhesion for example.

Figure 6

(left) Advancing and receding contact angle measurements with the needle method (right) Advancing and receding contact angle and roll-off angle with tilting method

Dynamic contact angles and contact angle hysteresis for additional information

Dynamic contact angles and contact angle hysteresis are often measured as they offer additional information on the properties of the surface. Although static contact angle is a good measurement to evaluate the hydrophilicity/phobicity of the material surface, it always assumes that the deposited drop is in global energy minimum (and also only possible energy state at that surface). This means that if you would tilt the sample even a little bit, the droplet would instantly start to move on the sample as it would not be stable any longer and could not resist the force of gravity. This is only true on the totally homogeneous surface both in terms of roughness and chemistry. However, in reality, there are numerous examples of stationary drops even on vertical surfaces, such as windows or plant leaves. This indicates that there are several metastable states and the drop can be at any of the local energy minima within the hysteresis range [27]. This hysteresis range is called contact angle hysteresis and it can be measured through advancing and receding contact angle measurements.

Dynamic contact angles and contact angle hysteresis has not been utilized in biomaterial research extensively. However, new advanced materials, such as superhydrophobic surfaces [28], have found applications also in the biomedical field which will increase the use of these measurements in the future.

The dynamic contact angle by the Wilhelmy plate method has also been utilized for adsorption studies which will be briefly reviewed in the following sections.

There are three main methods for the dynamic contact angle measurements; needle method, tilting plate, and Wilhelmy plate which all have their own advantages and limitations.

Needle method

Dynamic contact angles are often measured with the so-called needle method. In this method, the needle is brought close to the sample surface and the liquid (typically water) is dispensed slowly out of the needle. As the liquid front advances on the sample surface, the advancing contact angle is measured. The liquid is then withdrawn back to the needle, and the receding contact angle is measured when the contact line is moving. The principle of the method is presented in figure 6.

In biomedical applications, the method has been utilized to develop medical textiles such as masks, scrubs, and, gowning that should ideally be resistant to body fluids like blood, urine, and sweat [29].

Tilting plate

In the tilting plate method, a drop is placed on the sample as in the standard sessile drop measurements. The sample is then slowly tilted with the tilting sample stage or the tilting cradle (i.e. the whole instrument is tilted). The image of the drop is recorded as the stage is being tilted. As the droplet starts to move, the advancing angle is measured at the front (i.e. the advancing) edge of the drop and the receding angle at the back (i.e. receding) edge of the drop. The difference between advancing and receding contact angles is contact angle hysteresis. In addition, the method gives a sliding or roll-off angle which is measured at the point the droplet starts to move. The principle of the method is presented in figure 6.

The method is typically relatively simple and quick to perform. However, the results need to be critically compared as the size of the droplet effect, especially on the sliding angle value.

In biomedical applications, the sliding angle measurement has especially been utilized for the study of blood repellent coatings [30]. Blood damage or hemolysis is a serious problem in medical blood pumps which has to lead to innovations to reduce the friction force experienced by the blood inside these devices [31]. Different types of superhydrophobic coatings are hoped to solve the problem. To study the properties of these coatings, the static contact angle is not able to provide enough information and thus the tilting plate method is utilized instead. The sliding angle, as well as contact angle hysteresis, give indications on the flow behavior of the fluid in the blood-contacting applications.

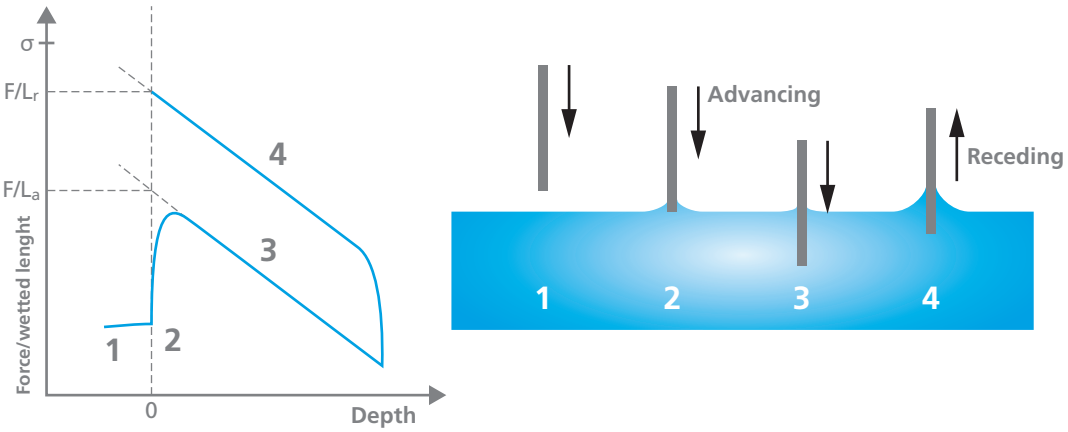

Figure 7
Advancing and receding contact angles with the Wilhelmy plate method

Wilhelmy plate

Wilhelmy plate measurement is based on a highly sensitive balance, where the sample is attached through the hook. The sample is immersed in the measuring liquid (most typically water), and the advancing angle is recorded. As the sample is emmersed from the liquid, the receding angle is detected (Figure 7). The difference between the advancing and the receding angle is contact angle hysteresis, which is automatically calculated. As the method gives always the dynamic contact angles, the results cannot be compared with those obtained with static methods. One advantage of the method is that the wettability is automatically measured on a large area. The method is also utilized to measure the wettability of thin fibers as the high sensitivity of the balance allows measurements of fibers down to 10 um in diameter.

For biomedical applications, the method is somewhat limited. The sample has to be of a regular size as the perimeter of the sample is used in contact angle calculation. Also, the surface finish has to be homogeneous on all sides of the sample as the contact angle is averaged over the immersed area.

For these reasons, the method is typically used in biomedical applications to measure samples like angioplasty ballons [32] where the perimeter of the sample can be easily determined. In some studies more complicated geometries, like dental screw implants, have been studied [33]. This requires a careful examination of the implant structure to obtain the wetted length for the measurements.

Adsorption studies present another interesting application of the Wilhelmy plate method [34]. The method is based on the change in advancing contact angle (and contact angle hysteresis) caused by the protein adsorption. In practice, the sample with the known perimeter is immersed in the protein solution. Several dipping cycles are done and the contact angles are measured. If the protein adsorption occurs, the advancing contact angle decreases due to hydrophilization of the surface caused by the protein adsorption. The method has been evaluated and compared with QCM (quartz crystal microbalance) measurements with a good correlation [35]. The method has been utilized to study both wanted [36] and unwanted protein adsorption [37, 38].

Conclusions

Wettability is one of the key parameters in biomaterial surface evaluation. Contact angle measurements are used to study the wettability of the materials. There are several different methods to measure contact angles which have been reviewed in this white paper and are also listed in table 1.

The selection of the measurement method is based on the sample being measured but different methods will also give slightly different information on your sample. The sessile drop measurement is a quick and easy way to determine the hydrophilicity/-phobicity of the sample. Roughness corrected sessile drop takes the surface roughness into account. Surface free energy can give additional information on surface chemistry and interactions with the surrounding liquids. Roughness corrected surface free energy gives surface energy values closer to the real chemical nature of the substrate. Dynamic contact angle measurements are especially useful when evaluating new, advanced materials for biomedical applications.

Both optical and force tensiometers range from manual to fully automated instrument. Optical tensiometer is, however, the typical choice for biomedical applications as all the measurement types are possible with the instrument. Furthermore, modular optical tensiometers can be updated with additional features such as roughness measurements, or picoliter dispensers if the need for those arises later.

MEASUREMENT	WHAT IS MEASURED?	INSTRUMENT	WHEN TO USE?
Sessile drop	Static contact angle	Optical tensiometer	Evaluation of hydrophilicity/-phobicity of the material, quality control
Roughness corrected sessile drop	Roughness corrected (static) contact angle	Optical tensiometer with 3D topography module	Evaluation of wettability of rough surfaces, surface treatment evaluation
Captive bubble	Static or advancing/receding contact angle	Optical tensiometer	Contact lens, hydrogels, other hydrated materials
Picoliter drops	Static contact angle	Optical tensiometer wihf picoliter dispenser	Contact angle measurements on a small surface area
Surface free energy	Static contact angle	Optical tensiometer	Evaluation of surface free energy, chemistry of the substrate
Roughness corrected surface free energy	Roughness corrected (static) contact angle	Optical tensiometer with 3D topography module	Evaluation of surface free energy of rough surfaces, surface treatment evaluation
Needle method	Advancing and receding contact angle and contact angle hysteresis	Optical tensiometer	Advanced, blood-repellent materials
Tilting plate	Advancing and receding contact angle and contact angle hysteresis, roll-off angle	Optical tensiometer with tilting stage or tilting cradle	Advanced, blood-repellent materials
Wilhelmy plate	Advancing and receding contact angle and contact angle hysteresis	Force tensiometer	Thin objects, objects with regular shapes, adsorption studies

Table 1

Overview of wettability measurement methods and their utilization in biomedical applications

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