AC 2007-2378: BIOMATERIALS SCREENING EXPERIMENT USING SESSILE DROP CONTACT ANGLES

Lanny Griffin, California Polytechnic State University

BIOMATERIALS SCREENING EXPERIMENT USING SESSILE DROP CONTACT ANGLES

I. Background: Biomaterials represent a unique class of materials that are specifically designed to be in contact with a living host. A biomaterial is any material – usually engineered, but it can be derived from living tissue – which interacts systemically with the living host. A biomaterial may be used in a surgical instrument or as an implant. To be considered as a biomaterial, the material must be biocompatible. Biocompatibility may simply be thought of as meaning the material must not harm the host (such as causing cancer or poisoning), nor can the host harm the material over the intended service life of the device (such as corrosion or fatigue). The body is an aggressive environment which will attempt to break down foreign materials. Some materials we want to be broken down over time, such as stitches, but more often we want the material to remain intact permanently, such as with joint replacements or pacemakers.

It is not possible at the present time to predict why some materials are biocompatible and others are not given the limited understanding the complexity of life. Currently, materials become admitted to biomaterials class by experimentation. Very few metals are biocompatible and therefore most metals are not biomaterials. On the other hand, many ceramics are biocompatible. *The body interacts with the material through the surface.* It has been estimated that the body cannot probe any more than 5 Å below the surface of any material. Therefore, it is not surprising that a lot of biomaterials research is focused on surface chemistry.

There are many sophisticated methods available to measure surface properties, but the simplest is to estimate surface properties by what is called a sessile drop test. That is, to put a small drop of a liquid on the surface of the material and directly measure the maximum angle the drop makes with the surface. The sessile drop test measures what is called the surface tension. Many of us have observed this property by looking at the meniscus that forms at the liquid vapor interface when a liquid is poured into a container.

Figure 1: A partial free body diagram of interfacial surface tensions acting on a droplet on a surface.

The form of the droplet on the surface is the result of three forces that act on the water (Figure 1). When we balance the forces in the x-direction, we obtain the equation relating the three forces.

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos(\theta) \quad (1)$$

In Equation 1, the symbol γ represents the surface energy and the subscripts sv, sl, and lv are solid-vapor, solid-liquid, and liquid-vapor, respectively. We are interested in obtaining the solid-vapor interfacial energy. The liquid-vapor surface energy is a known quantity, and the angle is known from experimentation. The surface energy is found by measuring the droplet contact angle of different fluids of known liquid-vapor interfacial energy and plotting the data on a Zisman plot (Figure 2). It should be noted that the interfacial energy eg, γ_{sv} , are dependent on the vapor and solid phases present. That is to say, if the solid is in air, it will have a differing wetting angle than if the solid is in pure oxygen.

Figure 2: Zisman plot for determining the critical surface tension.

The critical surface tension, γ_c , is found extrapolating the data to the point where the contact angle is 0 ($\cos(0) = 1$). For this example, it is 35 dynes-cm⁻¹. It should be noted that while Figure 2 shows a linear relationship, it is quite possible for the relationship between between surface energy and $\cos(\theta)$ to be non-linear.

For biomaterials, the surface energy has been suggested as related to the degree of interaction of the material with the host. More specifically, those surfaces that have high surface energy tend to adhere to the host, whereas, those materials with low surface energy do not adhere. Clinically, it is important for materials not to cause clotting in applications where the material is in contact with blood. A few examples of such devices are artificial blood vessels, dialysis machines, or prosthetic heart valves.

Recent studies have found hydrophobic polymer coatings have good potential for preventing clotting in small diameter Dacron blood vessel grafts [1]. Hydrogels and combinations of hydrophobic and hydrophilic monomers are being investigated as a means to exactly match the polar-dispersive ratio forces of the most passivating proteins. This engineered surface may then induce even less clot formation. The benefit would be that individuals with permanent devices, such as a prosthetic heart valve, may not have to be on anticoagulant medication, such as warfarin (Coumadin™).

From a thermodynamics point of view, it has been suggested that the process of adhesion of cells from a liquid suspension onto solid substrates may be related to the free energy of the adhesion [2] (ΔG_{adh}).

$$\Delta G_{adh} = \gamma_{cs} - \gamma_{cl} - \gamma_{sl} \quad (2)$$

In Equation 2, the symbol γ represents the surface interfacial free energy and the subscripts, c, s, and l refer to cell, solid, and liquid, respectively. Compare Equations 2 and 1 – how are they similar? For net negative free energies (high γ_s), the conditions are favorable for cellular adhesion, whereas, for a net positive free energy (low γ_s), the conditions are unfavorable (Figure 3).

The cell-solid interfacial energy is dependent on the cell type and solid substrate, but it can be calculated as [2] ²

$$\gamma_{cs} = \gamma_c + \gamma_s - 2 \left[(\gamma_c^d \gamma_s^d)^{1/2} + (\gamma_c^p \gamma_s^p)^{1/2} \right] \quad (3)$$

Here the subscripts c and s refer to cell and solid, respectively; and the superscripts d and p represent long-range dispersive interactions and short-range polar interactions, respectively.

Figure 3: Interfacial free energy as a function of substratum energy [3].

Baier [4] described an hypothetical optimal *biocompatibility zone* (Figure 4). The non-adhesive zone (A in Figure 4) represents a zone of minimal interaction or hydrophobic solid surfaces, whereas and the adhesive zone (B in Figure 4) are hydrophilic. Note the similarities between Figure 3 and Figure 4. For both cases, more hydrophilic surfaces (high γ_s) favor adhesion, while hydrophobic surfaces do not; although the transition between adhesion and non-adhesion are slightly different.

Figure 4: Relationship between biocompatibility and the critical surface tension of a solid [3].

Notice from Equation 2, that for the net free energy of adhesion to be positive, the contribution of the cell-solid energy must be greater than the sum of the cell-liquid and solid liquid energies. Consequently, for surfaces with a very high cell-solid interfacial energy compared to the sum of the cell-liquid and liquid-solid, the cell is less likely to adhere to the surface. It should also be noted, however, that extremely hydrophilic surfaces (very high γ_s), such as high energy methacrylates or hydrogels, do not promote either [5]. It should be realized that the actual contribution of the surface energy to the terms in Equation 2 is dependent on the liquid and cell type, as illustrated in Equation 3. Equation 2 also neglects electrical charge interactions, e.g. ionic. So, we can assume that while the surface energy is important to cellular adhesion and appealing because of its simplicity, the process of cellular adhesion is more complicated than described here, although there is certainly some important information that we can learn from this type of study.

From a biocompatibility perspective, the definition of what is biocompatible is somewhat dependent on the device/application. For a device such as an artificial artery, the material should not activate adverse reactions, such as clotting, nor promote adhesion on the blood side. However, the outside of the device should attach to the surrounding tissue [3].

Consider the materials tetrahedron (Figure 5). For a biomaterials application, such as a heart valve or artificial blood vessel, the **performance** we might be interested in is a device in which the surfaces have a low *thrombogenic* (blood-clot forming) potential. As we know that factors such as processing or structure will affect the surface properties and ultimately the performance. For this lab, we will be investigating the surface properties of two polymers and how these properties can be altered by surface modification.

Figure 5: Materials tetrahedron.

This lab is designed to be applicable for an introductory materials engineering or biomedical engineering lab for undergraduates. We currently use it as one of eight laboratory exercises for a sophomore level materials engineering course.

II. Learning Objectives:

- Discuss advantages and disadvantages of surface energy calculation by contact angle measurement
- Define what a biomaterial is and why some classes of materials are more or less biocompatible than others
- Discuss possible sources of variation
- Mathematically fit experimental data to an equation to determine material properties
- Explore the effects of surface treatment on the hydrophobicity of surfaces.
- Apply basic statistics to assess the quality of data

III. Goal:

Experimentally determine the surface energy of a material and discuss how surface properties and surface modification could be used to design a biomaterial with a specific host response.

IV. Procedures:

As with many labs, students were informed of the risks associated with the laboratory and asked to sign a form indicating that they understand the associated hazards. With this lab, we provided all MSDS's and device equipment literature in the form of an appendix. We also ask that the students inventory all equipment and ensure that all equipment and supplies are present.

Since the lab is a biomaterials lab, we provided generally used polymeric samples made of ePTFE (Gortex ®) and polyethylene. All that is required is that the surface be reasonably smooth, so woven Dacron would not be a good choice for a material. To create a Zisman plot, we needed to have polar and non-polar fluids with which to use, the problem is the potential hazard associated with many commonly used fluids. The obvious choice for a polar fluid is de-ionized water. For the other fluids, we chose Methyl Salicate, Dipolyethylene Glycol (Cellosolve), 1-2 Propylene Glycol (30%) and Methyl Salicate.

To conduct this lab, there is needed a contact angle goniometer, which is essentially a controlled dispenser and a precision camera. A good research grade system will cost approximately \$12,000, and for the lab, and one group of three students works well for each piece of equipment.

While the equipment is quite robust, it is prudent to have students familiarized with the equipment. For this lab, we provided pictures that show the various controls for moving the stages (Figure 6) and leveling the device (Figure 7).

Figure 6: Controls for stage motion (a) x translation. CW forward, CCW Backward, (b) y translation. CW left, CCW right, and (c) z translation. CW up, CCW down.

Figure 7: Checking to ensure the device is level.

The equipment we purchased and have used for the past three years has a very nice software package that controls the dispensing of the fluid and measuring of the contact angle for the droplet. We provided some rudimentary instruction on how to use the software (Figure 8). Particular additional instructions to a device might be stage lighting, how to change syringes, and power supply.

Figure 8: Menu items and tool bars for the VCAOptimaS software showing the important items for this laboratory.

We found that it was useful to include an image which shows the correct starting position for the syringe tip before conducting the lab (Figure 9).

Figure 9: Image of the tip.

The “meat” of the lab is to create a sessile drop on the surface. It is imperative to note that the contact angle measurement technique samples approximately 3-20 Å of the surface and so students should wear gloves and keep your specimens clean as the oils, dirt, etc, from your fingers *will* affect measurements. Note too that static electricity can adversely affect results.

We found that dispensing 0.25 µl drop works well, and should result in a liquid bubble forming and attach to the tip (Figure 10). With more non-polar fluids, such as methyl salicate, the drop may “wick” partially up the tip due to capillary action. This is not a real problem, but may require an additional 0.25 µl to be dispensed.

Figure 10: The sessile drop attached to the tip.

To remove the drop, the students will *slowly* translate the stage with the material placed under the tip in the z direction until it makes contact with the drop. The drop should immediately attach to the surface leaving a nicely formed drop on the surface (Figure 11)

Figure 11: Final position of the drop with respect to the tip. Be sure you can see the edge of the sample.

At this point, all that remains is to calculate the contact angles. The students will collect the data. We provide a data table for the students to fill out (**Table 1**). Note here that we are using only three of the four fluids. Three should be considered a minimum, but four would be optimum if time permits. The students collect five data points for each surface and fluid. This reinforces the need for experimental replicates and allows for construction of error bars on the plots. The data calculations are written on a table we provide (Table 2) which are ordinates and abscissas for the Zisman plot (Figure 2)

Table 1: Data table for the experiments.

		Left and Right Contact Angles (degrees)				
<i>Sample</i>	<i>Fluid</i>	<i>Trial 1</i>	<i>Trial 2</i>	<i>Trial 3</i>	<i>Trial 4</i>	<i>Trial 5</i>
<i>LDPE</i>	<i>Water</i>					
	<i>Cellosolve</i>					
	<i>Propylene Glycol</i>					
	<i>Methyl Salicate</i>					
<i>PTFE</i>	<i>Water</i>					
	<i>Cellosolve</i>					
	<i>Propylene Glycol</i>					
	<i>Methyl Salicate</i>					
<i>SM LDPE</i>	<i>Water</i>					

Table 2: Reduced data for the Zisman Plot.

<i>Sample</i>	<i>Fluid</i>	γ_v (dynes $\cdot$ cm ⁻¹)	θ_{Av}	θ_{SD}	$\cos(\theta_{Av})$	$\cos(\theta_{Av} \pm \theta_{SD})$	
<i>LDPE</i>	<i>Water</i>	71.99					
	<i>Cellosolve</i>	44.77					
	<i>Propylene Glycol</i>	55.00					
	<i>Methyl Salicate</i>	39.22					
<i>PTFE</i>	<i>Water</i>	71.99					
	<i>Cellosolve</i>	44.77					
	<i>Propylene Glycol</i>	55.00					
	<i>Methyl Salicate</i>	39.22					
<i>SM LDPE</i>	<i>Water</i>	71.99					

Sessile Drop Test of the Surface Modified Polymer

As an additional option, the polymer can be surface modified. We have a hand-held plasma generator. This portion of the lab allows students to directly measure the effect of surface modification on the surface tension of polyethylene. For the surface modified polymer, we have the students estimate the surface energy of the polymer using the water contact angle.

$$\gamma_s = \frac{1}{2}(\cos(\theta) + 1)\left(72 - \frac{\theta}{8}\right) \quad (3)$$

In Equation 3, θ is the contact angle of the water droplet in degrees.

The students report the experimentally found value and compare it to the literature values shown in Table 3, reporting the percentage of error calculated as:

$$\%error = \left(\frac{\gamma_{exp} - \gamma_{rep}}{\gamma_{rep}} \right) \times 100 \quad (4)$$

Where γ is the surface energy, and the subscripts, exp and rep are experimental and reported values, respectively.

Using the data in Table 2, the students calculate and report the average surface energy (Ave $\pm$ SD) of the LDPE, unmodified, compared to the plasma treated surface (Ave $\pm$ SD) and create a bar chart (Figure 12).

Figure 12: A bar graph comparing the effects of surface modification on surface energy.

Table 3: Critical surface tensions for some common polymers.

Material	Critical surface tension (dynes/cm)
Polytetrafluoroethylene	19
Poly(dimethyl siloxane)	24
Poly(vinyl fluoride)	28
Polyethylene	31
Polystyrene	33
Poly(hydroxyethyl methacrylate)	37

V. Discussion. Overall, the lab has been conducted for approximately three years with very positive student feedback. The lab is very effective in reinforcing statistical concepts and experimental techniques, such as cleanliness. There are occasionally issues with very hydrophobic surfaces (some forms of Gortex®) which can skew results. The water droplet may not attach to the surface in these cases, or static electricity can affect the results. To obtain the best results, it is important to ensure cleanliness of test specimens and that the working fluids are not contaminated. For students, it is gratifying to see first hand that their experimental results are in line with reported values. The surface modification can be considered as an optional portion of the lab, but it is valuable to help students understand how surface properties can vary as the result of a surface modification technique. Considering how biocompatibility is known to be related to surface energy (Figure 3, Figure 4), students can readily appreciate how the biologic interaction can be changed by altering the nature of the surface. Students also have commented how their perceptions about surface chemistry and biomaterials have been changed as a result of their brief exposure to these concepts.

This lab has been successful at our institution, and we believe that other institutions could benefit from this type of lab. This particular lab was designed to replace a Charpy Impact Test lab with something a little more state-of-the-art. The reinforcement with statistics helps students with data presentation and analysis.

VI. References

1. Biomaterials: Principles and applications, Ed by Park JB, and Bronzino, JD. CRC Press, Boca Raton, FL.
2. Schakenraad, JM; Busscher, H.J.; *et al.* (1988) Thermodynamics of cell spreading on solid substrata. *Cell Biophysics* **13**:75-91.
3. Biomaterials Science: An Introduction to Materials in Medicine. Ed by Ratner, B.D.; Hoffman, A.S.; Schoen, F.J. (1996) ; and Lemons, J.E. Academic Press, San Diego, CA
4. Baier, R.E. (1975) Applied chemistry at protein interfaces. *Adv Chem Ser* **145**:1.
5. Horbett, T.A; Schway, M.B. (1988) Correlations between mouse 3T3 cell spreading and serum fibronectin adsorption on glass and hydroxyethylmethacrylate-ethylmethacrylate copolymers. *J Biomed Mat Res* **22**:763-793.
6. Kolluri, O.S.; Kaplan, S.L.; and Rose, P.W. (1988). Paper presented at the Society of Plastics Engineers Advanced Polymer Composites '88 Technical Conference, Los Angeles, CA, November 1988