Water and the acute biological response to surfaces

ERWIN A. VOGLER *

Becton Dickinson Research Center, 21 Davis Drive, Research Triangle Park, NC 27709-2016, USA

Received 18 December 1998; accepted 15 April 1999

Abstract—Molecular self association in water through hydrogen bonding is a powerful organizational force leading to a three-dimensional hydrogen-bonded network (water structure) that profoundly influences solvent properties. Localized perturbations in the chemical potential of water as by, for example, contacting with a solid surface, induces compensating changes in water structure that can be sensed tens of nanometers from the point of origin using the surface force apparatus (SFA) and ancillary techniques. These instruments reveal attractive or repulsive forces between opposing surfaces immersed in water, over-and-above that anticipated by continuum theory (DLVO), that are attributed to a variable density (partial molar volume) of a more-or-less ordered water structure, depending on the water wettability (surface energy) of the water-contacting surfaces. Water structure at surfaces is thus found to be a manifestation of hydrophobicity and, while mechanistic/theoretical interpretation of experimental results remains the subject of some debate in the literature, convergence of experimental observations permit a quantitative definition of the heretofore relative terms 'hydrophobic' and 'hydrophilic'. In particular, long-range attractive forces (< 100 nm) are detected only between surfaces exhibiting a water contact angle $\theta > 65$ deg (defined as hydrophobic surfaces with pure water adhesion tension $\gamma^0 = \gamma^0 \cos \theta < 30$ dyn cm$^{-1}$ where $\gamma^0$ is water interfacial tension = 72.8 dyn cm$^{-1}$). Short range repulsive forces (< 5 nm) are detected between surfaces exhibiting $\theta < 65$ deg (hydrophilic surfaces, $\gamma^0 > 30$ dyn cm$^{-1}$). These findings together with other lines of chemical evidence suggest at least two distinct kinds of water structure and reactivity: a relatively less-dense water region against hydrophobic surfaces with an open hydrogen-bonded network and a relatively more-dense water region against hydrophilic surfaces with a collapsed hydrogen-bonded network.

Solvent properties of interfacial water profoundly influence the biological response to materials in a surprisingly straightforward manner when key measures of biological activity sensitive to interfacial phenomenon are scaled against water adhesion tension $\gamma^0$ of contacting surfaces. Protein adsorption, activation of blood coagulation, and bioadhesion are offered as examples in point, illustrating that the hydrophobic/hydrophilic contrast in the biological response to materials, often disputed in biomaterials science, is very clear when viewed from the perspective of water structure and reactivity at surfaces.

Key words: Water structure; biomaterials; biological response; surface energy; hydrophobic; hydrophilic; surface forces.

*Present address: Department of Materials Science and Engineering, Steidle Hall, The Pennsylvania State University, University Park, PA 16802-5005, USA.
1. INTRODUCTION

"Clearly other interactions precede protein adsorption: water adsorption and possibly absorption (hydration effects, ion binding and electrical double layer formation, and the adsorption and absorption of low molecular weight solutes — such as amino acids). The protein adsorption event must result in major perturbation of the interfacial boundary layer which initially consists of water, ions, and other solutes."

J. Andrade and V. Hlady, 1986 [1]

Water is the most unusual chemical I have encountered over the course of my 25-year career as a professional chemist. Relative to other compounds of similar molecular weight (such as nitrogen, methane, ammonia, and hydrogen sulfide) water has unusually high heat capacity, interfacial tension, and dielectric permittivity as well as exceptionally high melting, boiling, and critical temperatures. The viscosity of water actually decreases with increasing temperature (see references [2, 3] for compilations of water properties). Water contracts on melting, a rare property among all other known substances, reaching a maximum density at 3.98°C. This, together with an isothermal compressibility minimum observed near 50°C, suggests that liquid water has an open or expanded structure with an almost rigid character.

With the exception of a 4-year tour-of-duty in surface spectroscopy, my work has always involved water in some way, starting as a technician in a water testing laboratory through to my current role as a scientist in the biomedical device industry. Even the experience in high-vacuum spectroscopy, where the surface world is desiccated upper palatine, riveted my attention to water and taught the important lessons that: (i) water is the universal biological solvent, not vacuum; and (ii) an understanding of how water interacts with surfaces is a necessary prerequisite to a full appreciation of the role of surface chemistry in the biological response to materials.

This lesson that vacuum is not a biological solvent is apparently not such an obvious one as it may seem on the face of it. One frequently finds in biomaterials literature, almost universally in fact, that water is implicitly regarded as a neutral carrier of biology that does not participate in the biological response to materials [4–6]. Interestingly, this clearly unwarranted simplification is frequently preceded by an explicit acknowledgment of an important role for water in biomaterials. The introductory quotation is one such explicit articulation of the role of water in biomaterials, taken from an excellent, but now somewhat dated, review of protein adsorption prepared by Andrade and Hlady in 1986 [1]. Little fault can be found with this statement, or the general content of this excellent review for that matter, except that this collection of words is an exceptionally glib statement of some very complicated physical chemistry that occurs when water contacts a surface. It is so telegraphic, in
fact, that little guidance is provided as to how one is to incorporate these facts into a comprehensive view of the biological response to materials.

This contribution to the Symposium on Non-Fouling Surface Technologies attempts to more fully incorporate the current understanding of water structure and reactivity into an evolving paradigm for the biological response to materials. The content arises from a review of recent literature on water [6] drawn from disparate literature sources, ranging from chemical physics to molecular mechanics; all interpreted in terms of biomaterials and biocompatibility. This overview strongly suggests that water structure and reactivity drives the acute biological response to materials. The term 'acute' is applied herein to those biological events that transpire directly after a surface is contacted with a biomaterial (e.g. protein adsorption, cell attachment, cascade activation, etc.), but does not necessarily include chronic biological responses (e.g. tissue integration or lack thereof, inflammation, etc.) induced by long term contact.

2. A PARADIGM OF WATER INTERACTION WITH BIOMATERIALS

Scheme 1 interprets the mechanistic content of the introductory quotation by Andrade and Hlady [1] in the form of a descriptive chemical equation. This equation starts with the contact of a water-insoluble surface with an aqueous biological milieu and ends with a biological response to this surface. Scheme 1 decomposes a complex panoply of events that transpire at the interface into a few generalized chemical reactions that, in all likelihood, are much more concerted than indicated in this simple scheme. Nevertheless, Scheme 1 is useful in organizing

![Diagram of water interaction with biomaterials](https://via.placeholder.com/150)

Scheme 1. Paradigmatic view of the interaction of a surface with a generalized aqueous biological milieu identifying 'cause' and 'effect' in biocompatibility, leading from the interaction of a surface with water through to the observed biological response to materials. Formation of a 'water structure' vicinal to the surface effectively mediates all downstream events and is thus the only step in the interaction amenable to manipulation through surface modification.
hypotheses regarding separate mechanisms involved. The following sections provide some discussion of the individual steps involved, leading to the conclusion that an understanding of water structure and reactivity is essential to a mechanistic understanding of biocompatibility.

2.1. Step 1 — surface hydration

The first step in Scheme 1 is the instantaneous formation of a definitive water structure vicinal to the surface that results from the chemical reaction of water with surface-resident functional groups. Two important key words here are 'water structure' and 'chemical reaction'. As will be elaborated in subsequent paragraphs, water structure is a sort of code word or short-hand moniker referring to the solvent properties of water near a solute or surface (vicinal water) that strongly depend on the state of self association of vicinal water. Chemical reactions that constitute the enthalpic and entropic parts of the overall free energy of hydration include changes in hydrogen bonding and the concomitant changes in vicinal water density.

2.2. Step 2 — formation of a dynamic interface

The second step in Scheme 1 involves interaction of the various dissolved components of the biological milieu including proteins with the vicinal water region formed in the preceding water-contact step. This biological interface has been purposely termed 'dynamic' to emphasize that certain interfacial events such as adsorption are time dependent and reversible to some degree, leading to the exchange of different proteins (e.g. the Vroman effect [7–15]), ions, and water molecules. This region is hardly a stagnant region that can be frozen, freeze dried, or replicated in any way [5]. The interfacial chemistry that mediates the biological response to materials is created by the interaction of the various interactive components, including water, and does not exist when these components are separated or altered. Returning to the rudimentary lessons learned from high vacuum spectroscopy mentioned in the Introduction, dry-state chemistry is not the primary driver of biocompatibility and the role of the aqueous phase with dissolved proteins can not be summarily dismissed. A more accurate view of the acute biological response to materials must include the dynamic structure associated with hydration, formation of water structure, and possibly protein adsorption [4–6]. These are the essential elements that underlie the summary assessment embodied in the introductory quotation of Andrade and Hlady.

2.3. Step 3 — potentiation of the biological response

The third reaction in Scheme 1 involves the relatively slower steps among those represented in Scheme 1 such as zymogen activation or cell contact with a surface. These steps must be slower because of time-consuming interactions with allosteric proteins or ions and mass transfer steps associated with bringing these
items proximal to a surface. However, Step 3 embodies the observable events generally recognized as the biological response to materials and include cell/tissue adhesion and triggering of immune or inflammatory responses. In Scheme 1, the term ‘holistic’ is purposefully chosen to emphasize that this biological response to materials embraces all of the events that transpire within an interphase that separates bulk solid from bulk liquid, involving cells, proteins, ions, and importantly water.

2.4. Testable hypotheses and biophysical mechanisms

The primary value of Scheme 1 is the delineation of an unambiguous biophysical mechanism. Unlike the introductory words of Andrade and Hlady or related statements that can be found elsewhere in the biomaterials literature, Scheme 1 is an unambiguous proposition of cause-and-effect. The cause of the biological response to materials is the formation of a dynamic interfacial region. And while all of the steps leading to this response may be amenable to comprehension, none of these steps can be directly manipulated because, according to Scheme 1, water structure controls all downstream events. The only step that can be manipulated is the first one — the interaction of water with a surface. It is suggested from this water-oriented perspective that synthetic or surface-modification strategies toward the goal of achieving ‘biocompatibility’ for various biomedical applications should be directed toward control of water reactivity at interfaces rather than toward control of secondary biological responses that are only indirect consequences of surface chemistry. That is to say, the synthetic orientation should be focused on water, the universal biological solvent system, not exclusively on biological solutes such as proteins or suspended particles such as living cells. The destiny of these latter moieties near a surface is substantially controlled by interfacial water.

Also important are one or more hypotheses that can be derived from Scheme 1. These hypotheses can be put to test and the relevance of Scheme 1 to real biological interactions with surfaces better assessed, provided that some of the key parameters involved in formation of water structure can be identified. Toward this, it is useful to examine some of the leading chemical properties of water that might be involved in biological events at surfaces.

3. SOME ESSENTIAL PROPERTIES OF WATER

Table 1 compiles some important properties of water. Interested readers are directed to reference [6] for a more detailed discussion and complete citation of supporting literature than can be given here. The following text works through Table 1 in descending row order, leading to the summary conclusion that water self association is the predominating property controlling water behavior at surfaces. Section 4 will review two important experiments that probe this behavior of water at surfaces and begin to paint a picture of how water structure and reactivity influences
Table 1. Water properties and surface interactions

<table>
<thead>
<tr>
<th>Property, attribute, or physical phenomenon</th>
<th>Physical implication</th>
<th>Physical boundary conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water self associates through hydrogen bonding: 2H bond donors (Lewis acid) 2H bond acceptors (Lewis base) (3 kcal mol⁻¹, 10 ps associated lifetime)</td>
<td>Extended 3D network of self associating molecules. A transient ‘structure’</td>
<td>Crystalline ice — fully self associated Liquid water — transient self association</td>
</tr>
<tr>
<td>Water solvent properties (chemical potential) controlled by extent of self association</td>
<td>Less associated = more available H bonds (more reactive, more dense) More associated = fewer available H bonds (less reactive, less dense)</td>
<td>Water vapor — not self associated</td>
</tr>
<tr>
<td>Self association vicinal to ‘hydrophobic’ solutes and extended surfaces is perturbed</td>
<td>Solvent properties of vicinal water change to accommodate solutes or surfaces. Partial molar volume near hydrophobic surfaces increases (density decreases) to equilibrate vicinal water with bulk water</td>
<td>Small hydrocarbon solutes — Hydrophobic effect, Water orientation Macroscopic surfaces — Frustrated H bonding, ‘Dangling’ H bonds</td>
</tr>
<tr>
<td>Lewis acid/base sites on ‘hydrophilic’ surfaces compete with vicinal water self association</td>
<td>Surface functional groups ‘titrate’ vicinal water solvent properties. Partial molar volume near hydrophilic surfaces decreases (density increases) to equilibrate vicinal water with bulk water</td>
<td>Ion hydration — Lyotropic (Hoffmeister) series Surface hydration — Mechanism of water wettability</td>
</tr>
</tbody>
</table>

the biological response to materials. Section 5 will show through examples how these water properties are correlated with the acute biological response to materials.

3.1. Self association

The physicochemical basis for many, if not all, of water’s unusual properties is self association (row 1 column 1). Each water molecule comes complete with two hydrogen bond donors (Lewis acids, the protons) and two hydrogen bond acceptors (Lewis bases, the two un-shared pairs of electrons on oxygen). Each water molecule can thus associate with up to four nearest neighbors, or possibly
six in the event of hydrogen bond bifurcation [2]. These association reactions are not covalent chemical bonds with tens of kcal/mole bond strength but instead are relatively weak 3–5 kcal mol\(^{-1}\) associations (see, for example, reference [16]). As it turns out, this bond strength is approximately the same as the energy transferred from one molecule to another by collisions at room temperature, so hydrogen bonds are quite transient in nature, persisting only for a few tens of picoseconds [17]. Modern molecular models of water suggest that something between 75–80% of liquid-phase water at room temperature is associated with three or four neighboring water molecules, and thus this propensity to form a 3D network throughout the bulk phase is quite extensive (molecular dynamic models include lattice, free-volume, and multi-state theories; see reference [2] for an excellent review of computational methodologies). So extensive, in fact, that the term ‘structure’ is ubiquitous in the literature [18]. Historically, there have been two basic conceptualizations of bulk water structure: uniformist and mixture models (water structure concepts have been categorized and sub-categorized at different levels of detail; see references [17, 18] for more discussion). Although water-structure concepts have been largely superseded by various molecular dynamics models, these outmoded ideas will be briefly mentioned here as a means of focusing on the specific meaning of structure in a self-associating liquid to make the point that popular notions of ossified regions in water with ‘ice-like’ structures and ‘melting’ of water structure near surface are at best poor mental constructs and, at worst, completely misleading.

The uniformist (also termed continuum) model proposes that water molecules have more-or-less the same properties throughout the bulk water phase whereas the mixture model has it that water is comprised of more than one water species in dynamic equilibrium [18–22]. Said more precisely, the uniformist model proposes that water molecules are transiently interconnected through a hydrogen-bonded network characterized by a distribution of bond energies and geometries. The mixture model proposes discrete molecular species with different levels of hydrogen bonding [23]. One such mixture model is the ‘flickering cluster’ model of Frank and Wen [24, 25] in which it is proposed that extensively-hydrogen-bonded clusters co-exist in a sea of less-associated water. In the time-average limit, all molecules of bulk water have the same properties — the activity or chemical potential of bulk water must be uniform at equilibrium. At any particular instant in time, however, the mixture model has it that there are different species of water differentiated from one another on the basis of the extent of hydrogen bonding with other water molecules. Or said another way, there are different species of water differentiated on the basis of self association.

However viewed, water structure is controlled by self association through hydrogen bonding. At the extremes, fully-self-associated water is represented by a highly ordered 3D network of hydrogen bonds, like that obtained in crystalline ice (see Table 1, rows 1 and 2, column 3) whereas there are few hydrogen bond linkages in water vapor. The steady-state ‘structure’ of water between these extremes
is controlled by a transient self-association through hydrogen bonding. This transient nature of hydrogen bonding greatly weakens the utility of a water structure concept [17] as it might be practically applied by a chemist. These spatial relationships between molecules (structure) in water (and other liquids) persist only a few tens of picoseconds at most and are thus not defined on a practical temporal scale (line widths of infrared and Raman spectra set a lower limit to the lifetime of a hydrogen bond to no less than several vibrational periods of an intermolecular bond, about $2 \times 10^{-13}$ s; see references [17, 26] for more discussion). On the one hand, association reactions are sufficiently slow that statistical mechanics seems tantalizingly applicable, especially toward understanding the detailed chemical mechanism of water solvency. On the other hand, entanglements invited by adherence to a fixed paradigm of water structure in the bulk phase or near surfaces can be quite distracting. For biomaterial surface scientists interested in consequences of vicinal water structure on the biological response to materials, the thermodynamic average solvent property is probably most important, and least confusing to an already complex biochemical process, so long as it is borne in mind these solvent properties are mediated by water self association and that self association can manifest itself in a more-or-less organized group of molecules with what amounts to be a structure; albeit a very transient one.

3.2. Water solvent properties

Solvent properties of water (row 2, column 1 of Table 1) are directly connected to the extent of self association. This can be understood from the simple standpoint that more Lewis acid and base sites (herein generally referred to as ‘Lewis sites’ unless a distinction between acid and base is required) are available in less-associated water compared to that available in more-associated water. Available Lewis sites are reactive and it thus follows that less-associated water has a greater chemical potential than more-associated water. Interestingly, more-self-associated water must be less dense (greater partial molar volume) than less-self-associated water because formation of more-or-less linear hydrogen bonds takes up space and thus increases free volume (as in formation of crystalline ice; see Table 1, rows 1 and 2 of column 3). The ability of water to expand and contract (partial) molar volume with commensurate changes in chemical potential is an interesting mechanism that allows water to accommodate the presence of solutes and surfaces while maintaining an extensive hydrogen bond network [6].

3.3. Solvent properties of water vicinal to ‘hydrophobic’ solutes and surfaces

Imposition of a large hydrophobic solute or surface into water interrupts self association and thereby can alter solvent properties of vicinal water (row 3 column 1 of Table 1; hydrophilic/hydrophobic terminology will be quantitatively defined in subsequent sections). Generally speaking, it can be anticipated that water structure near hydrophobic solutes and surfaces must be different than in bulk water.
Molecular simulations suggest that water substantially maintains the 3D hydrogen-bond network in the local vicinity of small hydrocarbons (such as methane [2, 27–31]). This preservation of the maximum number of hydrogen bonds with nearest neighbors has an entropic cost, however, due to lost orientational flexibility vicinal to the hydrocarbon solute. Apparently, there are no structural ‘ice bergs’ around small hydrocarbons [32] as has been widely believed in the past (see Berendsen’s 1967 review, for example [17]) but imposition of the solute clearly imposes configurational constraints on self association. This propensity to maintain a self-associated state even near hydrophobic solutes is the underlying physical mechanism of the so-called hydrophobic effect.

The hydrophobic effect is related to the insolubility of hydrocarbons in water and is fundamental to the organization of lipids into bilayers, the structural elements of life as we know it [33, 34]. The hydrophobic effect is sometimes erroneously attributed to the strong association of hydrocarbon molecules through a form of the simila similibus solvuntur or the ‘like-likes-like’ principle of solubility and miscibility, possibly first articulated by Irving Langmuir himself in 1917 [35]. Actually, energetics of hydrocarbon-hydrocarbon interaction are approximately the same as water-hydrocarbon interaction, as has been pointed out by Irving Langmuir’s contemporary William D. Harkins [36], and much later in the 1970s by Joel Hildebrand [37] and Charles Tanford [33, 34]. Hydrocarbons are sparingly soluble in water due to the strong self association of water, not the strong self association of hydrocarbons, and entropy of hydrophobic hydration is an important contributor to the overall free energy of hydration as well as a route to better understanding the ‘microscopic realities’ [38] of water near hydrophobic solutes.

Difficulties in maximizing hydrogen-bonded nearest neighbors are exacerbated near extended hydrophobic surfaces relative to that occurring near small solutes because water cannot effectively reorient around such a surface. An end effect of this frustrated hydrogen bonding is the ‘dangling hydrogen bonds’ that have been theoretically predicted [39] and spectroscopically resolved from hydrogen bonds in bulk water [40–42]. As a result, water vicinal to a hydrophobic surface must be momentarily at a state of a higher chemical potential than bulk water — a circumstance that can not prevail at equilibrium. At fixed temperature, pressure, and mole fractions of all constituents including water, the only means to equilibrate vicinal water with bulk water is through a compensating increase in partial molar volume (decreased density).

3.4. Solvent properties of water vicinal to ‘hydrophilic’ solutes and surfaces

Lewis sites on ‘hydrophilic’ solutes or surfaces can effectively compete with self association of water (row 4, column 1 of Table 1). For ionic solutes, the familiar lyotropic or Hofmeister series that measures the extent of ion hydration results. The lyotropic series also sequences the extent of ion adsorption to surfaces, apparently following the same general rules as any other solute; the more hydrated the ion, the less likely it is to adsorb to surfaces [6]. This must be important in enzyme
reactions at surfaces because highly-hydrated ions such as Ca\(^{2+}\) and Mg\(^{2+}\) have powerful allosteric effects.

Surface density of Lewis sites on an extended macroscopic hydrophilic surface ‘titrate’ vicinal water solvent properties. As an example, water adsorbed to Lewis acid and base sites on ultra-dispersed diamond treated with oxygen and nitrogen have been spectroscopically resolved and differentiated from adjacent water molecules hydrogen-bonded to these surface-adsorbed water molecules [43]. According to this view then, water solvent properties (structure) near surfaces can be thought of as a sort of continuum. At the perfectly hydrophobic end of this continuum, surfaces bear no surface-resident Lewis sites and interact with water only through ‘dispersion’ or van der Waals forces (relatively less-dense vicinal water; see row 3 Table 1). At the fully water-wettable end of the continuum, surfaces bear a sufficient population of Lewis sites to completely erode water structure, leading to complete water wetting (relatively more-dense vicinal water; see row 4 Table 1). Of course, these ideas fall in the mechanistic category of water wetting phenomenon (row 4 column 3 of Table 1). One might expect that purely ‘Lewis base surfaces’ are different than purely ‘Lewis acid surfaces’ in this regard and however these may be defined; with water molecules accepting electron density from electron-rich surface functional groups (such as oxygen in ether or ester linkages for example) through water hydrogen atoms and donating electron density to electron-deficient surface functional groups (such as protons on hydroxyl groups for example) through water oxygen atoms. This kind of interaction would effectively orient water ‘protons down’ on electron-rich surfaces and ‘protons up’ on electron-deficient surfaces with potentially different effects on vicinal water solvent properties. Perhaps surface acidity/basicity for practical biomaterial problems might best be defined according to the extent and nature of competition with vicinal water self association. At the extreme of water–surface interactions, water may become ionized in contact with certain surfaces that fully abstract protons or hydroxyl groups from water.

Ordering of water near water-wettable surfaces has long been anticipated (see, for example, Drost-Hansen’s 1969 review [44]) along with the strong electrostatic fields that would result from aligning water dipoles at the surface (see references [45, 46] for more discussion). Satisfactory representation of ideas about water structure near water-wettable surfaces is far outside the intended scope of this writing, so let it simply be concluded that the behavior of biology at water wettable surfaces — ranging from protein adsorption [47] to blood contact properties [48–50] to cell/tissue adhesion [51] — must be profoundly influenced by water solvent properties vicinal hydrophilic surfaces.

4. TWO EXPERIMENTS IN WATER BEHAVIOR AT SURFACES

The essential properties of water summarized in Table 1 provide more-or-less circumstantial chemical evidence for variable water structure and solvent properties
at surfaces linked to the extent of self association. Neither this circumstantial evidence nor cited spectroscopic observations provides clues regarding the distance this putative vicinal-water region extends from a water-contacting surface into the bulk-water region. Likewise, no direct evidence is provided that relates the thickness of vicinal water to contacting surface chemistry/energy. Perhaps the best physical evidence for variable water solvent properties at surfaces arises from the surface force apparatus and measurement of condensate water films that form on carefully controlled quartz surfaces. This evidence is itself extremely controversial [6], but it is worthwhile to review this important work here as a means of illuminating the behavior of water at surfaces anticipated by the preceding sections.

4.1. Surface forces

The surface force apparatus is a tool that has emerged from the liquid-phase physics laboratory that can measure forces between objects brought into close proximity with nanometer resolution. The essential elements of the modern version of the surface force apparatus were introduced by Tabor, Winerton, and Israelachvili (see, as examples, references [52–54]) but various instrumental attempts date back as far as 1928 (see reference [55] for a recent and complete historical review and the introduction of reference [56]). This device has confirmed the general predictions of DLVO so long as the opposing surfaces are separated by air or other structureless fluids (DLVO is the standard theory of colloid science founded on the principle that repulsive electrostatic forces and attractive dispersion forces between two objects separated by a suspending medium but in close proximity are additive. To a first approximation, the attractive forces follow an inverse power law with distance while repulsive forces are exponential, so that net inter-surface forces depend strongly on separation distance. DLVO is termed a continuum theory because the formulation of the double-layer and dispersion force terms assume that the medium separating opposing objects is structureless, defined solely by its bulk dielectric permittivity).

One of the very surprising, and presumably informative, observations arising from the surface force apparatus is that of long-range ($\leq 100$ nm) attraction between opposing hydrophobic surfaces immersed in water, over-and-above that anticipated by DLVO. These excess, so-called ‘hydrophobic’ forces are observed to decay exponentially in separation distance $D$ with a characteristic decay length $D_0$ having the form $\exp(-D/D_0)$. On occasion, surface force data are fit with the sum of two exponential decays [57] having the form $[\exp(-D/D_1) + \exp(-D/D_2)]$, where $D_1$ is the characteristic decay length of short-range forces (typically less than 1 nm) and $D_2$ is the characteristic decay length of longer-range forces (typically not greater than 40 nm). However interpreted, the origin of hydrophobic forces has been a_1 mystery since the initial observations were made by Israelachvili and Adams[58, 59]. The exact physical mechanism of hydrophobic forces is controversial [60–62] but some theories trace the origin of these forces to what are essentially density (structural) fluctuations of water captured between opposing hydrophobic surfaces in close proximity [63–68]. Presumably, surface forces
(between opposing poorly-water wettable surfaces immersed in water) do not follow the predictions of DLVO because water is not a structureless continuum fluid insensitive to the local environment but instead accommodates contact with surfaces by varying the extent of self association or, equivalently, water structure.

Figure 1 summarizes a single set of experiments selected from many (see reference [6] for a recent review) measuring hydrophobic forces performed by Yoon et al. using a modified atomic force microscope (AFM) as a kind of surface force apparatus [69]. In these elegant studies, a silane-treated glass sphere with a water contact angle \( \theta = 109 \) deg glued to the AFM tip was brought into close proximity (in pure water) to a silica plate made more-or-less water wettable by silanization (Fig. 1 insert) using the cantilever system of the AFM. Either attractive (hydrophobic) or repulsive (hydration) forces, over-and-above that anticipated by DLVO, were measured depending on the water wettabiliy of the silica plate. Measured characteristic decay length for various plate wettabilities are plotted in Fig. 1 from which it is evident that \( D_0 \) decays linearly with \( \tau^0 \) over a range \(-40 < \tau^0 < 30 \text{ dyn cm}^{-1} \) as \( D_0 = [-0.544 \pm 0.027] \tau^0 + [18.12 \pm 0.41] \) \( (R^2 = 98.74\%) \); it should be noted that water wettabiliy of the silica plate reported in Fig. 1 are converted from the

![Diagram](image-url)

**Figure 1.** Monotonic decrease in the characteristic decay length of surface forces \( D_0 \) with water adhesion tension \( \tau^0 \) observed using an atomic force microscope (AFM) as a surface force apparatus [69]. Insert illustrates the physical set-up in which the cantilever of the AFM is depicted as a simple rod bearing a glass sphere rendered hydrophobic by silanization (extensively adapted from [6]). The cantilever was used to bring the glass sphere to within close proximity to a silica plate surface with nanometer resolution. Silica plate surface wettability was varied by partial silanization and reported on the paired abscissas as either contact angle \( \theta \) or adhesion tension \( \tau^0 \). Note that extrapolation of the linear trend through attractive hydrophobic forces crosses \( D_0 = 0 \) very close to the limit of detectable hydrophobic forces suggested by Berg et al. [70] and is annotated as the 'BECB limit'.

'equilibrium contact angles' $\theta$ reported by Yoon et al. [69] to adhesion tension $\tau^0 = \gamma^0 \cos \theta$; where $\gamma^0 = 72.8$ dyne cm$^{-1}$ for pure water). Somewhere within the range $20 < \tau^0 < 40$ dyne cm$^{-1}$ attractive (hydrophobic) forces deviate from the monotonically-decreasing trend and become repulsive (hydration) forces, although there is insufficient data to determine exactly how this occurs. However, extrapolation of the linear trend to $D_0 = 0$ suggests that hydrophobic forces are not supported on surfaces more wettable than $\tau^0 = 33.7$ dyne cm$^{-1}$ ($\theta = 62.4$ deg), in close agreement with the 65 deg limit suggested by Berg et al. [70] (see 'BECB limit' annotations in Fig. 1). Clearly, water-wettability of opposing surfaces strongly influences the manner by which attractive and repulsive forces are propagated through water. Additional insights into the behavior of water at surfaces more wettable than the BECB limit can be obtained by comparison of surface force measurements to experimental results obtained through independent means, such as the formation of condensate films discussed in the next section.

### 4.2. Condensate films

Figure 2 illustrates experiments performed by Pashley and Kitchner [71] nearly two decades prior to the work of Yoon et al. In these carefully-performed studies, equilibrium thickness of 'condensate' water films grown from vapor onto crystalline

![Diagram](image)

**Figure 2.** Sharp increase in condensate film thickness formed on quartz surfaces with increasing $\tau^0$ formed from water vapor at nearly 100% relative humidity [71]. Insert illustrates the physical set-up where the quartz specimen is contained in a water-vapor cell in such a way that water-film thickness could be interrogated with an ellipsometer (extensively adapted from [6]). Figure annotations including the 'BECB' limit are reproduced from Fig. 1. Note that measurable water film thickness could be detected only on surfaces more wettable than the BECB limit.
quartz plates was determined by ellipsometry (see Fig. 2 insert). Quartz surface wettability was controlled using rigorous cleaning, heat dehydroxylation, and methylation. Figure 2 plots observed condensate water film thickness against quartz-plate water wettability wherein reported contact angles have been once again converted to $\tau^0$ assuming $\gamma^0 = 72.8 \text{ dyn cm}^{-1}$ for direct comparison to Fig. 1. It is quite startling that very thick ($\leq 150$ nm) water films are supported on fully-wettable quartz surfaces ($\tau^0 \rightarrow 72.8 \text{ dyn cm}^{-1}$). Water film thickness sharply decreases as surface water-wettability decreases ($18 < \tau^0 < 70 \text{ dyn cm}^{-1}$). Condensate films are occasionally discounted as microdroplets, but this possibility has been eliminated by Pashley et al. Also, formation of thick condensate water films have been confirmed by other investigators [72], finding that the Kelvin equation correctly predicts the equilibrium vapor pressure of water confined in a wedge between fused-silica surfaces, provided that condensate film thicknesses of the order reported by Pashley are taken into account. Thick water films cannot be silicic acid gels because there is no chemical or spectroscopic evidence that hydrated silicon oxides are much thicker than 1–2 nm [73–77]. Thus, Pashley and Kitchner provide seemingly incontrovertible evidence for multilayer water adsorption, corroborating work of others on the same subject extending back as far as 1887 (see citations in reference [78]).

Water molecules in direct contact with fully water-wettable quartz surfaces are adsorbed with an adhesion tension $\tau^0 = \gamma^0(\cos 0 \deg) = 72.8 \text{ dyn cm}^{-1}$. Israelachvili points out that this bound water layer has little to do with water–water (self association) interactions at wettable surfaces but rather is completely dominated by surface–water (Lewis site) interactions [45]. It is this water–surface interaction that deprives bound-water molecules from a nearest-neighbor hydrogen-bonded association. Somehow this binding energy propagates into subsequent layers, supporting up to 150 nm-thick, free-standing condensate films comprised of something of the order of 600 water molecules.

The mechanism of energy propagation favored by Pashley and Kitchner is long-range double-layer repulsion, although this mechanism was not interpreted in terms of water structure or state-of-association. Perhaps the condensate film is comprised of water molecule layers with alternating oriented dipoles like that described by Israelachvili [45, 46] in which water molecules stack in an asymmetric or ‘staggered’ arrangement, with the surface-bound layer in a hydrogen-atom-down configuration (for an electron-rich surface, net dipole pointed up, see Section 3.4) and subsequent layers alternating dipole direction. Note that this hypothetical arrangement defeats water self association throughout the condensate film in a manner that would not permit propagation of hydrophobic forces through the formation of low-density water mentioned in Section 3.2. Through whatever mechanism of formation, this remarkable formation of thick water films on wettable surfaces bears further consideration in the context of the surface force work discussed in the previous section because, taken in conjunction, surface forces and
condensate films suggest at two main types of water behavior against two classes of surfaces that will be defined as hydrophobic and hydrophilic in the next section.

4.3. A quantitative definition of hydrophilicity and hydrophobicity

Comparison of surface force data of Yoon et al. (Section 4.1) to the work of Pashely and Kitchener (Section 4.2) shown in Fig. 3B suggests existence of at least two types of water structure and reactivity at surfaces. Surfaces less water wettable than the BECB limit support hydrophobic attractive forces (as measured by $D_0$ in Fig. 1) but do not support condensate water films (as determined by ellipsometry in Fig. 2). Free-standing condensate water films of measurable thickness can be obtained on surfaces more wettable than the BECB limit that propagate repulsive hydration forces but not attractive hydrophobic forces.

Interpreted in terms of Besseling’s body-centered cubic lattice model of water in contact with a surface [68], which only considers hydrogen bonding (self association) effects, one is lead to the conclusion that water within the confines of a gap between opposing surfaces less wettable than the BECB limit is less dense than bulk water and propagates attractive forces (row 3 of Table 1). Water in a gap between surfaces more wettable than the BECB limit is oriented by hydrogen-bonding with these surfaces in a manner leading to repulsive forces. According to the discussion of Section 3.4, it is also anticipated that water vicinal to wettable surfaces is more dense than that of surrounding bulk water (row 4 of Table 1).

The BECB limit thus represents an exquisite balance between self association and interaction with opposing surfaces that leads to a water density and orientation equivalent to bulk water. Interpreted in terms of the self association of water discussed in Sections 3.2 to 3.4, increasing the surface density of Lewis sites on increasingly water-wettable surfaces erodes self association (increases density or decreases partial molar volume; row 4 of Table 1) through competitive bonding with the surface, leading to a reduction in the attractive surfaces forces observed between purely hydrophobic surfaces (row 3 of Table 1). Surfaces more wettable than the BECB limit substantially erode water structure, leading to a collapsed hydrogen-bonded network and repulsion between opposing surfaces.

Figure 3B suggests a quantitative basis for the definition of the terms ‘hydrophobic’ and ‘hydrophilic’ [6] that are frequently applied as only relative terms with significant scientist-to-scientist variation in technical usage [79]. Hydrophobic surfaces are proposed to be defined as those supporting hydrophobic forces and are less water wettable than the BECB limit (for surfaces of the kind represented in Figs 1 and 2 $\tau^0 < 30 \, \text{dyn cm}^{-1}, \theta > 65 \, \text{deg}$) whereas hydrophilic surfaces are proposed to include those that do not support hydrophobic forces and are more wettable than the BECB limit ($\tau^0 > 30 \, \text{dyn cm}^{-1}, \theta < 65 \, \text{deg}$). Alternative definitions based on theoretical considerations that warrant citation herein [80] place the hydrophobic/hydrophilic dividing line [81] at $\tau^0 \approx 45 \, \text{dyn cm}^{-1}$ ($\theta \approx 52 \, \text{deg}$).
Figure 3. (A) Relationship connecting the biological response to materials with (B) water structure and reactivity at biomaterial surfaces inferred from a composite view of surface forces (Fig. 1) and formation of condensate water films (Fig. 2). According to the perspective represented in (B), at least two distinct kinds of water structure and reactivity are formed: a relatively less-dense water region against surfaces less wettable than the BECB limit with an open hydrogen-bonded network and a relatively more-dense water region against surfaces more wettable than the BECB limit with a collapsed hydrogen-bonded network. This anticipates in (A) two basic kinds of biological response to materials and a quantitative definition of hydrophobicity and hydrophilicity; a "Type I" biological response to hydrophobic surfaces defined as less water wettable than the BECB limit and "Type II" biological response to hydrophilic surfaces more water wettable than the BECB limit. Figure annotations including the 'BECB' limit are reproduced from Fig. 1.
5. WATER SOLVENT PROPERTIES IN THE BIOLOGICAL RESPONSE TO MATERIALS

Scheme 1 asserts that water structure and reactivity vicinal to surfaces effectively mediates the biological response to materials. Figure 3B suggests from experiment that at least two kinds of water structure and reactivity are supported against surfaces of varying water wettability: water against hydrophilic surfaces (as defined in the preceding section) lying on the right of the BECB limit and water against hydrophobic surfaces lying on the left side of the BECB limit. A combined interpretation of Scheme 1 and Fig. 3B suggests that there must be two types of biological response to materials, yielding the sharp hydrophilic/hydrophobic contrast in biomaterials posited in Fig. 3A.

Figure 3A is proposed as a paradigm for the acute biological response to material surfaces that bifurcates at the BECB limit into 'Type I' and 'Type II' responses corresponding to hydrophobic and hydrophilic surfaces as defined herein, respectively. The following sections illustrate this bifurcation in the acute biological response to materials using a number of examples ranging from protein adsorption, activation of the blood coagulation cascade, to blood clot and cell adhesion. Although these examples cover a broad range of in vitro observations of the biological response to material surfaces, it should be noted that most of the examples presented are drawn from my personal research experience, supplemented with only a few results drawn from other researchers that provide the necessary data permitting correlation of the biological response to materials (dependent variable) to the water-wettability $r^0$ of the surface under study (independent variable). More complete discussion can be found in [6]. Also, it is noteworthy that these experiments employ glass and silicon bearing self-assembled monolayers (SAMs), polymers, and surface-treated polymers that do not observably swell in water. These surfaces bear greater-or-lesser surface densities of ‘electron rich’ functional groups that probably hydrogen bond to water substantially through electron-donation (see row 4 of Table 1), although experimental means of verifying this are not routinely available. It may be speculated, therefore, that the sharp hydrophilic/hydrophobic contrast in the biological response to materials represented in these experimental examples and the graded response between these extremes in water wettability may be substantially related to the ‘titration’ of vicinal water solvent properties by increasing surface densities of electron-rich surface functional groups (see row 4 of Table 1). Otherwise, if a similar set of surfaces bearing electron-deficient functional groups were studied in a similar manner, a different pattern may emerge (see Section 3.4). Practically speaking, given the preponderance of oxidized surfaces in nature, one might expect the pattern represented in Fig. 3 to be more the rule than exception.
5.1. Protein adsorption

There can be no doubt that solute adsorption from water, in general, and protein adsorption in particular, is as technically challenging as it is scientifically important. Adsorption is basic to fields of commercial interest including adhesives, agriculture, biochemistry, biomaterials, biology, biotechnology, chemistry, colloids, composites, detergents, electronics, and many more. In biomaterials, the protein adsorption literature is controversially symmetric; for every ‘factoid’ there seems to be a counterbalancing ‘anti-factoid’, for every yin there is an equally-compelling yang. This results, in part, from a profusion of analytical technique defying inter-experiment interpretation and from a reckless inter-comparison of surface, interface, and interphase compositions that are essentially not comparable. Interested readers are directed to reference [6] for a detailed discussion of these points including issues related to adsorption equilibria.

For the general purpose of this proceedings summary and for the elaboration of the controlling role of water in protein adsorption, let it be sufficient to comment that a need exists for in situ methods that measure protein concentration within an interphase separating the bulk liquid phase from a bulk solid phase. These in situ methods should permit direct and unambiguous correlations with surface energetics and be applicable to chemically-undefined biological milieu of everyday practical interest of biomaterial practitioners. A useful tool that meets some of these needs is the ‘adsorption map’ approach that preserves much of the power of Gibbsian thermodynamics but is not in any way restricted to situations in which solution composition must be defined. Equally important, the adsorption map is a means of relating adsorption to \( \tau^0 \) and is therefore directly connected to the structure and reactivity of water at surfaces. Adsorption map theory has been discussed in detail elsewhere [82] and only a few salient features need to be reiterated here so that the reader can readily interpret adsorption maps and appreciate the revealed relationship between \( \tau^0 \), protein adsorption, and the reactivity of water at surfaces.

An adsorption map is a graphical construction formed by plotting the domain of an adsorption index \([\tau' - \tau^0]\) against the range of surface wettabillity in pure water \( \tau^0 \) (or buffered saline as appropriate herein; see Fig. 4A as an example). The adsorption index \([\tau' - \tau^0]\) compares the adhesion tension \( \tau' \) measured with a chemically-undefined surfactant or protein mixture (having characteristic interfacial tension \( \gamma' \)) to the adhesion tension \( \tau^0 \) obtained with pure water or saline (having characteristic interfacial tension \( \gamma^0 = 72.8 \text{ dyn cm}^{-1} \)). The adsorption index is sensitive to adsorption because adsorbed solute causes measurable changes in \( \tau' \) and, consequently, \([\tau' - \tau^0]\).

According to adsorption map theory, \([\tau' - \tau^0] > 0\) is characteristic of surfaces that support adsorption whereas surfaces that do not support adsorption are characterized by \([\tau' - \tau^0] \leq 0 \text{ dyn cm}^{-1}\). Boundaries of the map are derived from surface thermodynamics (the Young equation) and enclose all observable data for a particular protein or surfactant system in what is termed an adsorption triangle (see Fig. 4A, for example). Area of an adsorption triangle falling on the map where
Figure 4. (A) Adsorption map for EDTA anticoagulated porcine plasma [82]. The adsorption index \( [r' - r^0] > 0 \) dyn cm\(^{-1}\) for surfaces that support protein adsorption and \( [r' - r^0] < 0 \) for protein repellent surfaces. Note that the data trend line (dashed) crosses \( [r' - r^0] = 0 \) near \( r^0 = 37 \) dyn cm\(^{-1}\) near the BECB limit (see Fig. 3) where also the propensity to activate the coagulation cascade (B) just begins a transition from a relatively flat dependence on surface energy (as measured by \( r^0 \)) to an exponential-like increase with surface energy. Comparison of results (A) to the paradigm of the biological response to materials represented by Fig. 3A suggests that protein adsorption is a 'Type I' biological response to materials. By contrast, comparison of results (B) to the paradigm of the biological response to materials represented by Fig. 3A suggests that activation of the plasma coagulation cascade is a 'Type II' biological response to materials. Closed and open symbols represent \( r^0 \) and \( r' \) determinations from advancing and receding contact angle measurements, respectively (see [96, 97] for an interpretation of advancing and receding contact angles in adsorption measurements).
\[ \tau' - \tau^0 \geq 0 \text{ dyn cm}^{-1} \] corresponds to surfaces supporting adsorption whereas area of the adsorption triangle falling below \([\tau' - \tau^0] = 0 \text{ dyn cm}^{-1} \) correspond to solute-repellent surfaces to which water strongly binds. Thus, the adsorption map is a relatively new tool in biomaterials surface science that allows straightforward characterization of the wetting behavior of heterogeneous and chemically-undefined biological fluids.

Adsorption maps have been prepared for surfactants drawn from non-ionic, anionic, cationic, and perfluorinated classes as well as for various purified proteins and whole blood serum proteins. In all of these circumstances \([\tau' - \tau^0] \) data corresponding to advancing and receding contact angle measurements fall along monotonic trend lines within the corresponding adsorption triangle [49, 82]. Consequently, only a few measurements on surfaces with different \(\tau^0\) are required to completely characterize the surfactant or protein system. Within this diverse set of test surfactants and proteins, it is generally found that interpolation of the trend line to \([\tau' - \tau^0] = 0 \text{ dyn cm}^{-1} \) occurs between \(30 > \tau^0 > 15 \text{ dyn cm}^{-1} \) (average \(\tau^0 = 26.8 \pm 6.8 \text{ dyn cm}^{-1} \) or \(\theta = 68.2 \pm 5.7 \text{ deg} \), in rough correspondence with the BECB limit (see Fig. 3; this statement is specific to surfactants that adsorb by mechanisms leading to a decreased contact angle and exclude surfactants that adsorb through mechanisms leading to an increased contact angle through a dewetting effect). This observation leads to the hypothesis that surfactant or protein adsorption is driven by the same water structure-reactivity sensed by the surface force apparatus that scales with \(\tau^0 \) in such a manner that hydrophobic surfaces \((\tau^0 < 30 \text{ dyn cm}^{-1})\) support adsorption from water whereas hydrophilic surfaces \((\tau^0 > 30 \text{ dyn cm}^{-1})\) do not support adsorption from water. Apparently, water is too strongly bound to hydrophilic surfaces to be displaced by solute through surface dehydration mechanisms. This latter supposition correlates with the formation of strongly-bound condensate water films illustrated in Fig. 2.

As an example that will have utility in the next section discussing the coagulation of blood, an adsorption map for EDTA-anticoagulated porcine blood plasma is shown in Fig. 4A [49], from which it is evident that porcine plasma proteins do not adsorb to surfaces more wettable than \(\tau^0 = 37 \text{ dyn cm}^{-1} \) corresponding to \(\theta_{\text{adv}} = 59 \text{ deg} \). Contact angle measurements employed in adsorption mapping of porcine plasma thus positively detect plasma protein adsorption to hydrophobic surfaces but not to hydrophilic surfaces.

Interpreted in terms of the paradigm of the biological response to materials represented in Fig. 3A, protein adsorption is a Type I biological response to materials.

5.2. Activation of the blood coagulation cascade

Figure 4B shows the exponential-like relationship between \(\tau^0 \) and the propensity to contact activate the blood plasma coagulation cascade obtained with procoagulant surfaces prepared from oxidized polystyrene film sections [48]. Comparison to the adsorption map for blood serum in Fig. 4A (see Section 4.1) leads to the
conclusion that the hydrophobic surfaces that support protein adsorption from water are inefficient procoagulants whereas protein repellent, hydrophilic surfaces are efficient activators of the blood plasma coagulation cascade. Evidently, enzymes of the blood plasma coagulation cascade exhibit very different activity at hydrophilic surfaces, especially the fully water-wettable variety, than at hydrophobic surfaces.

It is interesting to speculate that the catalytic potential of water-wettable surfaces is in some way related to the special solvent properties of water against these surfaces, especially as related to the distribution of ions briefly mentioned in Table 1. Possibly, the conversion of blood Factor XII to the proteolytic form Factor XIIa that triggers subsequent zymogen-enzyme interconversions of intrinsic pathway of blood coagulation does not occur through a formal surface adsorption step, as commonly depicted for contact activation [83–86], but rather through an encounter with an exotic ionic environment in close proximity to water-wettable procoagulant surfaces. This proposition reconciles adsorption map results and biochemical mechanisms as well as possibly explaining why hydrophobic surfaces, known to efficiently adsorb proteins, are not efficient procoagulants.

However mechanistically interpreted, experimental results of Fig. 4B correlating procoagulant catalytic potential with surface wettability $\tau^0$ viewed in light of the paradigm of the biological response to materials represented in Fig. 3A, suggests that activation of the blood coagulation cascade is a Type II biological response to materials distinct from protein adsorption which is a Type I response.

5.3. Blood clot adhesion

Figure 5 illustrates a hydrophilic/hydrophobic contrast in blood clot adhesion through a very simple experiment in which a polystyrene film, partly protected by two clean glass cover slips, was exposed to an oxidizing plasma sustained in

![Figure 5. Illustration of a simple experiment in blood clot adhesion wherein a portion of a polystyrene test strip is rendered partly hydrophilic by surface oxidation in an RF-driven oxygen plasma. The portion covered by two clean glass microscope slides is protected from the oxygen plasma and remains untreated (hydrophobic). The resulting test strip was subsequently immersed in whole coagulating porcine blood. After coagulation, the test strip was removed, triple rinsed with saline and the interface region between plasma-treated and untreated was examined using optical microscopy (see Fig. 6).]
a conventional parallel-plate, diode-type radio-frequency reactor. A reasonably sharp interface between surface oxidized (hydrophilic with advancing contact \( \theta = 0 \) deg) and untreated polystyrene (hydrophobic with advancing contact angle \( \theta = 90 \) deg) was thus created, permitting ready comparison of clot adhesion to these two distinctly different surface regions by bringing this polystyrene test strip in contact with coagulating porcine blood contained in an ordinary glass test tube. A similar visual experiment comparing blood clot adhesion to the inside surface of a hydrophobic polystyrene tube to that of a hydrophilic clean-glass tube can be easily performed by using an exogenous bolus of thrombin to induce rapid coagulation in both tubes; except of course microscopy is not as easily carried out.

After blood had thoroughly coagulated, the polystyrene test strip was removed from the clot, rinsed lightly by triple immersion into a saline wash, and examined under an inverted microscope. Figure 6 is a micrograph (400x magnification) of the interface region between surface oxidized and untreated polystyrene showing a tenaciously-adherent fibrin mat with several immobilized red blood cells in the hydrophobic region and no visible clot on the hydrophilic region.

Interpreted in terms of the paradigm of the biological response to materials represented in Fig. 3A, the above results suggest that blood clot adhesion is a Type I biological response to materials, almost undoubtedly associated with protein adsorption to the hydrophobic region of the polystyrene test strip. According to the adsorption map discussed in Section 5.1, no protein adsorbs to the hydrophilic region of the polystyrene test strip. It is indeed interesting that blood clot does not adhere to this water-wettable surface, at least as visualized by microscopy. Possibly, water associated with the hydrophilic portion of the test strip is so tightly bound (with an adhesion tension \( \gamma^0 = \gamma^0(\cos 0 \text{ deg}) = \gamma^0 = 72.8 \text{ dyn cm}^{-1} \)) that neither protein nor blood clot can effectively dehydrate the surface and form an adherent mat.

5.4. Mammalian cell attachment and adhesion

Attachment of mammalian cells to a surface from aqueous suspension typically follows a sigmoidal attachment profile in time, reaching a steady-state plateau that is sensitive to cell-surface compatibility [51, 87]. Adhesive surfaces exhibit significantly higher maximal percentage of cell inoculum attached (%\( I_{\text{max}} \)) than attachment-resistant surfaces. Figure 7 plots %\( I_{\text{max}} \) obtained for Madin-Darby Canine Kidney (MDCK) cells on a series of glass and polystyrene surfaces with varying water wettability \( \tau^0 \) [6].

As indicated by arbitrary trend lines drawn through the data, %\( I_{\text{max}} \) exhibits a sharp transition between \( 20 < \tau^0 < 40 \text{ dyn cm}^{-1} \), depending on whether advancing or receding \( \tau^0 \) is examined (see figure legend). Hydrophobic surfaces resist attachment of MDCK cells whereas hydrophilic surfaces promote cell attachment, reflecting a general trend (but not an exclusive trend) observed in the culture of cell in vitro [51] dating back at least four decades (see Introduction of [88]). It is of interest that surfaces made progressively more wettable than the BECB limit do not
Figure 6. Photomicrograph (400 ×) of blood clot adhesion to the interface region between plasma treated and untreated portions of the polystyrene test strip prepared as illustrated in Fig. 5. Note that no adherent clot mass is apparent in the oxygen-plasma-treated zone, whereas a tenaciously adherent clot mass was readily observed in the untreated zone. Comparison of clot adhesion to these two portions of the polystyrene test strip to the paradigm of the biological response to materials represented by Fig. 3A suggests that blood clot adhesion is a ‘Type I’ biological response to materials almost assuredly associated with protein (fibrinogen) adsorption to hydrophobic materials.
Figure 7. Steady-state maximal attachment (as percent of inoculum, %I_max) of Madin-Darby Canine Kidney cells (MDCK, epithelioid) to polystyrene and glass surfaces [6] with varying water wettability \( \tau^0 \) prepared by oxidative gas discharge or silanization [4, 82, 96, 97]. Comparison of results to the paradigm of the biological response to materials represented by Fig. 3A suggests that attachment of mammalian cells is a "Type II" biological response to materials. Figure annotations including the "BECB" limit are reproduced from Fig. 1. Closed and open symbols represent \( \tau^0 \) determinations from advancing and receding contact angle measurements.

yield progressively higher level of attachment efficiency. An interpretation is that a critical surface density of Lewis sites is required to optimize cell attachment beyond which additional surface density does not measurably impact attachment efficiency.

It is also of interest to note in the context of these data that the adsorption map for fetal bovine serum proteins (FBS, 10% in Dulbecco’s Modified Eagle Medium) used in the culture of these cells indicates that surfaces more wettable than \( \tau^0 = 24.4 \pm 3.7 \, \text{dyn cm}^{-1} \) (advancing) do not support adsorption of proteins from this heterogeneous biological milieu [82]. According to this point of view, MDCK cells attach to protein-repellent surfaces (\( \tau^0 > 30 \, \text{dyn cm}^{-1} \)) but not to surfaces that support protein adsorption (\( \tau^0 < 30 \, \text{dyn cm}^{-1} \)). Interpreted in terms of the paradigm of the biological response to materials represented in Fig. 3A, this suggests that mammalian cell adhesion is a Type II biological response to materials. This observation parallels the propensity of hydrophilic surfaces to contact activate the blood plasma coagulation cascade (discussed in Section 5.2) but not the adhesion
of blood clots (discussed in Section 5.3). Clearly the adhesive mechanism for blood clots is quite different from the adhesive mechanisms for mammalian cells; the former associated with the adsorption of protein and the latter not associated with the adsorption of protein.

Similar relationships among cell attachment, cell spreading, and $\tau^0$ can be found in other literature sources as well. The interested reader is directed to reference [6] for a more detailed discussion.

5.5. Microbial cell adhesion

It can be anticipated at the outset that the attachment and adhesion of mammalian cells should be very different than the attachment and adhesion of microbial cells. After all, mammalian cells are derived from tissues where materials such as ceramics, metals, and polymers are entirely foreign. Furthermore, protein composition in mammalian system is quite well conserved in nature and the overall chemical constitution of mammalian cell membranes is quite different from that of microbes. In sharp contrast to mammalian cell natural history, evolutionary forces on naturally-occurring microbes, persevering as individuals or colonies, must have at least partly influenced the ability to adhere to both organic and inorganic materials. Clearly, the chemical constitution of the various environments where microbes are found varies considerably.

Thus, it is not surprising to find that microbial cell attachment is much more complex than mammalian cell attachment, but here too sharp transitions in attachment occur at surface wettability near the BECB limit. Figure 8 reproduces work of Fletcher and Pringle [89] relating microbial (Aeromonas hydrophilia H22 and Acinetobacter sp. H3) attachment from artificial seawater to the wettability of plastic and glass surfaces. Interestingly, microbial attachment to surfaces increases sharply near the BECB limit (at least for Acinetobacter) and does not clearly favor hydrophobic surfaces over hydrophilic surfaces. And unlike the attachment of mammalian cells shown in Fig. 7 in which cell attachment is negligible on hydrophobic surfaces with $\tau^0 < 0$ dyn cm$^{-1}$, thousands of microbial cells attach per-unit-area to these surfaces, suggesting that control of microbial fouling by manipulation surface energy alone may not be possible, especially in light of the prodigious rate at which microbial cells replicate.

Other microbial strains exhibit different attachment patterns on surfaces with varying water wettability. For example strains of Pseudomonas, Arthrobacter, and E. coli [90] and ‘soil bacteria’ [91] are found to exhibit a sigmoidal attachment profile on surfaces with varying water contact angle (not shown) with more cells attached to hydrophobic surfaces and less on hydrophilic surfaces. Here too, however, an inflection in attached cell number is noted near the BECB limit ($\theta \approx 65$ deg). Similarly, Hsieh and Timm [92] find a polar attachment response to hydrophobic and hydrophilic surfaces for Staphylococcus aureus, as shown in Fig. 9.
Figure 8. Microbial cell (A. hydrophila H22 and Acinetobacter sp. H3) adhesion to plastic and glass surfaces [89] relating attachment from artificial seawater to the wettability of plastic and glass surfaces. Interestingly, attachment of these microbes to tested surfaces peaks within the vicinity of BECB limit but a clear attachment preference to hydrophobic or hydrophilic is not observed. Figure annotations including the 'BECB' limit are reproduced from Fig. 1.

6. CONCLUSIONS

"...efforts in many branches of molecular biophysics and biochemistry will prove fruitless unless the structure of water and its changes is considered a possible factor of importance in biochemical behavior."

Herman Berendsen, 1967 [17]

This summary of my contribution to the Symposium on Non-fouling Surface Technologies ends where it began — with a quotation supporting the need to explicitly incorporate the role of water in our evolving understanding of the biological response to materials. The important factors that must be incorporated are that (i) water is a very small molecule with (ii) a propensity to self associate through transient hydrogen bonding which (iii) controls the overall solvent properties of
water in such a way that (iv) vicinal water solvent properties are altered by the mere presence of solutes or surfaces.

Hydrophobic surfaces tend to promote self association whereas Lewis acid/base sites on hydrophilic surfaces tend to erode self association by competition with hydrogen bonding. These surface-induced alterations of water association can be sensed by the surface force apparatus and suggest that there is a reasonably sharp bifurcation in water properties occurring at what has been termed herein as the ‘BECB Limit’. In particular, long-range attractive forces are detected only between surfaces exhibiting a water contact angle \( \theta > 65 \) deg (with pure water adhesion tension \( \tau^0 = \gamma^0 \cos \theta < 30 \) dyn cm\(^{-1}\) where \( \gamma^0 \) is water interfacial tension = 72.8 dyn cm\(^{-1}\)). Repulsive forces are detected between surfaces exhibiting \( \theta < 65 \) deg (\( \tau^0 > 30 \) dyn cm\(^{-1}\)). These findings suggest at least two distinct kinds of water structure and reactivity: a relatively less-dense water region against hydrophobic surfaces with an open hydrogen-bonded network and a relatively more-dense water region against hydrophilic surfaces with a collapsed
hydrogen-bonded network. This bifurcation in water properties also suggests an experimental basis against which the heretofore only qualitative terms 'hydrophilic' and 'hydrophobic' can be quantified. Hydrophobic surfaces are defined herein as those that support hydrophobic forces and are less water wettable than the BECB limit ($\tau^0 < 30 \text{ dyn cm}^{-1}, \theta > 65 \text{ deg}$) whereas hydrophilic surfaces do not support hydrophobic forces and are more wettable than the BECB limit ($\tau^0 > 30 \text{ dyn cm}^{-1}, \theta < 65 \text{ deg}$).

Solvant properties of water vicinal to surfaces profoundly influence the acute biological response to materials in a surprisingly straightforward manner when key measures of biological activity sensitive to interfacial phenomena are scaled against water adhesion tension $\tau^0$ of contacting surfaces. As examples, hydrophobic surfaces ($\tau^0 < 30 \text{ dyn cm}^{-1}$) support adsorption of various surfactants and proteins from water because expulsion of solute from solution into the interphase between bulk solid and solution phases is energetically favorable. Adsorption to hydrophobic surfaces is driven by the reduction of interfacial energetics concomitant with replacement of water molecules at the surface by adsorbed solute (surface dehydration). Hydrophilic surfaces ($\tau^0 > 30 \text{ dyn cm}^{-1}$) do not support adsorption because this mechanism is energetically unfavorable. Protein-adsorbent, hydrophobic surfaces are inefficient contact activators of the blood coagulation cascade whereas protein-repellent hydrophilic surfaces are efficient activators of blood coagulation. Mammalian cell attachment is a process distinct from protein adsorption that occurs efficiently to hydrophilic surfaces but inefficiently to hydrophobic surfaces. Thus, the hydrophobic/hydrophilic contrast in the acute biological response to materials, often disputed in biomaterials science, is very clear when viewed from the perspective of water structure and reactivity at surfaces.

Biomaterials is similar to any other branch of materials science in that practitioners seek, or at least could greatly benefit from, structure-reactivity relationships that form the essential rule base that expresses quantitative connections between material chemistry and the utility of that material in end use. Appropriateness of a material in various biomaterial applications, that is to say the biocompatibility of that material [5, 6], is dictated, or at least strongly influenced, by the acute biological response to those materials. As a consequence, biomaterial surface scientists have long sought the structure-function relationships that connect surface and interfacial chemistry with biocompatibility. These connections frequently implicate 'surface energy' of biomaterials as the primary driver of the biological response to surfaces, even though there are no biophysical theories derived from first-principle antecedents that predict such a relationship. An archetypal articulation of this surface energy concept arose from Baier et al. who proposed that Zisman's critical surface energy $\gamma_c$ was directly related to biocompatibility [93-95]. Baier's offering was followed by a cascade of these various surface energy and surface tension component theories making essentially the same proposition in different surface energy metrics. Experience clearly teaches that 'biocompatibility' is in some way connected to surface energetics but direct confirmation of these surface-energy re-
relationships has not been forthcoming in a compelling manner from nearly three decades of scrutiny. That is to say, no mechanistic rationale has been offered that explains purported relationships between surface energy and the biological response to materials.

This work suggests that the acute biological response to materials is not directly driven by ‘dry-state’ chemistry or energy but rather by the solvent properties of water vicinal to surfaces that, in turn, are responsive to surface chemistry and energy. Thus, the biological response to material surfaces is only secondarily related to surface chemistry as resolved in a high-vacuum spectrometer [5]. Two basic kinds of acute biological response to materials are resolved and associated with the two basic kinds of surfaces mentioned above: ‘Type I’ responses against hydrophobic surfaces which are distinct from ‘Type II’ responses occurring against hydrophilic surfaces.

Acknowledgements

Dr. Gary Harper is gratefully acknowledged for his experimental input on blood clot adhesion. The author is indebted to Professor Michael Paulaitis and Dr. Andrea Liebmann-Vinson for useful discussions of the manuscript. Ms. Linda Tingen is thanked for help in preparing figures.

REFERENCES