THERMODYNAMICS OF SHORT-TERM CELL ADHESION IN VITRO

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ABSTRACT A thermodynamic theory of short-term (<2 hr) in vitro cell adhesion has been developed which allows calculation of reversible work of adhesion and estimation of a term proportional to cell-substrate contact area. The theory provides a means of determining a parameter related to membrane wetting tension for microscopic cells that does not require special manipulations which might desiccate or denature delicate cell membranes. Semiquantitative agreement between predicted and experimentally-measured cell adhesion obtained for three different cell types (MDCK, RBL-1, and HCT-15) in two different liquid phase compositions of surfactants (Tween-80 and fetal bovine serum) supports concepts and approximations utilized in development of theory. Cell-substrate contact areas were largest for wettable surfaces treated with ionizing corona or plasma discharges and smallest for hydrophobic materials for each cell type studied. Contact area for the continuous dog-kidney cell line MDCK was larger than that of either the leukemic blood cell RBL-1 or the anaplastic human colon cell HCT-15.

INTRODUCTION

Biomaterials are artificial substances (polymers, ceramics, metals, etc.) that can be successfully used in a biological environment such as a living organism to treat, replace, or augment some tissue, organ, or function of that organism. Understanding cellular interactions with biomaterial surfaces is crucial to successful use in many applications. For example, strong cell adhesion and rapid proliferation on prosthetic devices is generally thought to promote incorporation of the device into a body (6–7,48,56). By contrast, circulating blood-born cells should not adhere to a vascular-graft material since platelet adhesion is thought to be a prerequisite for thrombus formation (6,33,43). At the same time, it would be beneficial for these grafts to become coated with the host's vascular endothelium which imparts a naturally nonthrombogenic surface (8,13). For most applications it would be desirable to have biomaterial surfaces that are nonadherent to bacterial cells since colonies of these organisms are responsible for pathogenesis of diverse states such as periodontal disease (17), osteomyelitis (28), and post-operative vascular-graft infectivity (21,46). Moreover, bacterial adherence has been implicated in the infection of incisional wounds (18), intravenous catheters (53), and intrauterine contraceptive devices (51).

A broader definition of biomaterials has been suggested that includes any material designed to replace, supplement, store, or otherwise come into intimate contact with living biological cells or biological fluids (9). Fitting under this definition are materials used in tissue-culture technology in which animal cells derived from some source organism are sustained in vitro. Plasticware, most commonly polystyrene, used in tissue culture is typically subjected to some proprietary chemical pretreatment by the manufacturer in order to make cells more adhesive to these surfaces (4,19). Although a great deal of work has been aimed at elucidating those surface functional groups responsible for cellular adhesion and optimizing surface treatments (15,19,30,41), adhesive mechanisms at the chemical level remain obscure and no single pretreatment method seems generally applicable (12).

Thus there is need in the art of biomaterial design a means of assessing utility of various materials for both in vivo and in vitro applications (7). In effort to fill this need, material scientists have attempted to correlate biomaterial surface properties with cell adhesiveness by applying the so-called DLVO theory of colloid stability (26). In this theory, adhesiveness is a balance between short-range electrostatic repulsion and longer-range attractive potentials between a macroscopic substrate and a microscopic cell or particle immersed in some specific liquid phase. Despite difficulties in determining accurate values for various cell parameters such as surface charge densities (16), application of DLVO has been remarkably useful in understanding the connection between material and cell science. A far less complex, alternative theory (but not totally independent from DLVO, 40) utilizes concepts of surface tensions and wetting to determine bioadhesiveness. Generally speaking, this approach attempts to calculate work of cellular adherence or equivalently, the free energy

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change of adherence from interfacial forces. Interfacial forces or tensions are, in turn, determined by application of standard and modified methods of surface physical chemistry (20,22–23). These concepts have been used to describe biological phenomena such as biocompatibility of prostheses (10,21), phagocytosis of bacteria by human neutrophil cells (57), platelet adhesion to polymers (35), and adhesion of bacteria to dental surfaces (17). Although DLVO and surface tension theories have been confirmed or corroborated by in vitro experiments utilizing cells in pure saline solutions, successful application to either more complicated protein-containing solutions or the physiological condition have been very limited, mainly due to complications introduced by adsorption of proteins from solution (36–37,59). Application of theory to these situations fails because changes in interactive potentials between cells, liquids, and substrates caused by adsorption have not been accounted for (40).

Toward a fuller understanding of the role of interfacial forces and effects of adsorption, cell adhesion to various plastic substrata from isotonic-salt solutions containing varying concentrations of detergent or proteins have been measured. Results demonstrate that adsorption effects on adhesion can be accounted for based on the surfactant action of the detergent or protein. A straightforward theory of cell adhesion has been developed which states that adhesivity is a balance of substrate and cell wetting tensions. Cell membrane wetting characteristics have been determined by applying theory to experimentally-measured cell adhesion.

GLOSSARY

$\gamma_{(ij)}$	Interfacial tension between mutually insoluble phases i,j. Subscripts i and j denote c = cell, s = solid, l = liquid, or v = vapor (ergs/cm^2)
$\gamma^0_{(ij)}$	Interfacial tensions at zero cell adhesion (ergs/cm^2)
$\theta_{(ij)}$	Contact angle between phases i,j (degrees)
$\Gamma_{(ij)}$	Interfacial excess (moles/cm^2)
$W_{(ij)}$	Reversible work of adhesion of phase i to phase j (ergs/cm^2)
$\tau_{(i)}$	Wetting tension of liquid l on solid phase i: $\tau_{(i)} \equiv \gamma_{(iv)} - \gamma_{(il)} = \gamma_{(lv)} \cos\theta_{(il)}$ (ergs/cm^2)
$\tau^0_{(i)}$	Wetting tension of liquid l on phase i at zero cell adhesion (ergs/cm^2)
$\Delta\tau_{(i)}$	Phase i wetting tension difference between substrates 1 and 2: $\Delta\tau_{(i)} \equiv \tau_{(i,2)} - \tau_{(i,l)}$ (ergs/cm^2)
$\Delta\tau^0_{(i)}$	Phase i wetting tension difference between substrates 1 at 2 at zero cell adhesion: $\Delta\tau^0_{(i)} \equiv \tau^0_{(i,2)} - \tau^0_{(i,l)}$ (ergs/cm^2)
$\tau^*_{(c)}$	Cell wetting tension parameter: $\tau^*_{(c)} \equiv \tau_{(c)} - \tau^0_{(c)}$ (ergs/cm^2)
$\Delta\tau^*_{(c)}$	Cell wetting tension parameter difference between substrates 1 and 2: $\Delta\tau^*_{(c)} \equiv \tau^*_{(c,2)} - \tau^*_{(c,1)}$ (ergs/cm^2)
Ω	Empirical parameter proportional to cell-substrate contact area (cm^2)
$A_{(ij)}$	Fitted parameters characterizing
$D_{(ij)}$	sigmoidally-shaped interfacial tension
$K_{(ij)}$	curves as a function of surfactant
$N_{(ij)}$	concentration
$Ln\ C$	Log surfactant concentration
$Ln\ C^0$	Log surfactant concentration at zero cell adhesion
G_T	Free energy of interaction between a microscopic cell and a macroscopic substrate (ergs/cm^2)
G_B	Free energy barrier B to adhesion (ergs/cm^2)
G_{SM}	Free energy of interaction at the secondary minimum SM (ergs/cm^2)
G_{PM}	Free energy of interaction at the primary minimum PM (ergs/cm^2)
N_T	Number of cells used in adhesion experiments
N_{SM}	Number of cells in SM
N_{PM}	Number of cells in PM
K	Cell adhesion equilibrium constant
k	Boltzman constant (erg/^{0}K)
R	Gas constant (erg/cm^2 · mole · ^{0}K)
I_A	Per cent of cell inoculum attached (%).

THEORY

Cell Adhesion Model

The adhesive process under consideration is deposition of a monodisperse suspension of mammalian cells (10–20 μm diameter) onto a planar, nondeformable substrate from a sessile liquid phase. The liquid phase is a physiological-saline solution containing some concentration of detergent or protein (surfactants). Gravity conveys suspended cells to within close proximity of the substrate-liquid interface where reversible adhesion can occur, as described in greater detail in subsequent sections. Cells are regarded as deformable spheres with a highly invaginated membrane surface coated with a polysaccharide "fuzz" (39). Adhesion is defined as a process leading to the formation of a cell-substrate interface and concomitant destruction of a cell-liquid and substrate-liquid interface. Cell-substrate contact area is assumed to be characteristic of cell and substrate surfaces but independent of liquid-phase composition because liquid interfaces are eliminated in the adhesion process.

Adhesional Work and Wetting Tensions

Work of adhesion for a micrscopic cell or particle to a macroscopic substrate is given by the Dupré equation (32, 40) which states that $W_{(sc)} = \gamma_{(cl)} + \gamma_{(sl)} - \gamma_{(sc)}$. $W_{(sc)}$ is the reversible work required to remove an adherent cell from a substrate, leaving cell and substrate in equilibrium with the surrounding liquid phase. Reversible work is a direct measure of the free energy change on adhesion, a quantity frequently encountered in the literature (1–2, 17, 34–36, 54–57). Interfacial tensions $\gamma_{(cl)}$ and $\gamma_{(sl)}$ are difficult, if not impossible, to obtain in proteinaceous liquid phases, though there have been a number of theoretical and experimental attempts (22–24, 34–37, 47, 57, 60).

Some of these difficulties can be avoided by recasting the Dupré equation in terms of wetting tensions for a substrate and cell in equilibrium with the same liquid phase. Wetting tensions are experimentally measurable, at least in principle, whereas individual interfacial tensions are not.

Substrate wetting tension is given by the Young equation (see, for example, 29); $\tau_{(s)} \equiv \gamma_{(sv)} - \gamma_{(sl)} = \gamma_{(lv)} \cos \theta_{(sl)}$ where $\theta_{(sl)}$ is the contact angle formed by a droplet of liquid with interfacial tension $\gamma_{(lv)}$ on a smooth, dry substrate. Both $\gamma_{(lv)}$ and $\theta_{(sl)}$ are easily measured. By contrast, contact angles on microscopic-cell membranes that are subject to desiccation and denaturation can not be directly determined. Nevertheless, for present purposes it is useful to define a hypothetical cell wetting tension $\tau_{(c)} \equiv \gamma_{(cv)} - \gamma_{(cl)} = \gamma_{(lv)} \cos \theta_{(cl)}$ by analogy to the substrate case. Eq. 1 results from substituting $\tau_{(s)}$ and $\tau_{(c)}$ for $\gamma_{(sl)}$ and $\gamma_{(cl)}$ in the Dupré equation, respectively.

$$W_{(sc)} = \gamma_{(cv)} + \gamma_{(sv)} - [\tau_{(c)} + \tau_{(s)}] - \gamma_{(sc)} \qquad (1)$$

It is noteworthy that this form of the Dupré equation contains (cv) and (sv) interfacial terms, despite the fact that these interfaces are not involved in the cell adhesion process. This results from an inconvenient choice of reference state which can be resolved by choosing a more relevant standard condition.

Experience has shown that for a particular cell type and liquid phase, test substrates can be selected such that no adhesion occurs at an empirically-determined biosurfactant concentration (see Results and Discussion section). In this very specific instance $W_{(sc)} = 0$, which occurs at some surfactant concentration designated $Ln\ C^0$. Wetting tensions evaluated at $Ln\ C^0$ are assigned the special symbols $\tau^0_{(c)}$ and $\tau^0_{(s)}$. Inserting these relationships into Eq. 1 for the condition of zero adhesion leads to the conclusion that $\gamma_{(sc)} = \gamma^0_{(cv)} + \gamma^0_{(sv)} - [\tau^0_{(c)} + \tau^0_{(s)}]$. It is presumed that $\gamma_{(sc)}$ is independent of liquid-phase composition as discussed previously. A simplified version of Eq. 1 in which vapor-phase interfaces do not appear can be obtained by substitution of $\gamma_{(sc)}$ and by making the approximations $\gamma^0_{(cv)} \simeq \gamma_{(cv)}$, $\gamma^0_{(sv)} \simeq \gamma_{(sv)}$. These approximations are valid for dilute surfactant concentrations which do not significantly alter vapor phase composition or partial pressure. Since only $\tau_{(s)}$ and $\tau^0_{(s)}$ terms are directly measurable, $\tau_{(c)}$ and $\tau^0_{(c)}$ remain unknown. In the event that $W_{(sc)}$ can be determined, however, the cell wetting-tension parameter $[\tau_{(c)} - \tau^0_{(c)}]$, which is assigned the special notiation $\tau^*_{(c)}$, can be determined from the resulting simplified version of Eq. (1).

$$\tau^*_{(c)} \equiv [\tau_{(c)} - \tau^0_{(c)}] = -[\tau_{(s)} - \tau^0_{(s)}] - W_{(sc)} \qquad (2)$$

Subsequently, cell surface excess can be calculated by application of Gibbs' relationship (5, 25, 29, 43, 50) since $\tau^*_{(c)}$ differs from the actual cell wetting tension $\tau_{(c)}$ only by the constant offset term $\tau^0_{(c)}$: $\partial[\tau^*_{(c)}]/\partial[Ln\ C] = \partial[\tau_{(c)}]/\partial[\mathrm{Ln\ C}] = RT[\Gamma_{(cl)} - \Gamma_{(cv)}]$. Positive values for the difference $[\Gamma_{(cl)} - \Gamma_{(cv)}]$ indicate concentration of surfactant at the cell membrane (cl) interface.

Work of Adhesion and Equilibrium Cell Adherence

The time course of cell adhesion to a substrate (attachment rate) from a sessile fluid phase can be divided into three distinct phases (27). First, there is a brief lag phase while cells gravitate to the substrate (31) and collect at an attractive potential well separated 5–8 nm from the surface by an electrostatic barrier (52, 61). This energy well is referred to as a secondary minimum with free energy of interaction G_{SM}. Thereafter, attached-cell number rapidly increases at some initial rate as cells penetrate the electrostatic barrier G_B and fall into a deeper, primary minimum G_{PM} at or near the interface. Initial-rate phase is followed by declining rate-of-attachment to a plateau or equilibrium-adherence level. Initial rates have been found to be sensitive to and dependent on electrostatic barriers to formation of close substrate contacts whereas equilibrium-adherence levels are controlled by short-range forces such as interfacial energies and formation of receptor-ligand complexes (34–36, 54, 58). In view of these findings and since adhesivity is related to reversible work of adhesion (32), it can be expected that experimentally-determined equilibrium cell adhesion is a direct measure of $W_{(sc)}$. Furthermore, it is proposed that equilibrium adhesion is dependent on the relative depths of G_{PM} and G_{SM}. In the event $G_{PM} \ll G_{SM}$ (deeper primary well), it can be anticipated that all cells would adhere so that $N_{PM} = N_T$. Conversely, if $G_{PM} \simeq G_{SM}$ then $N_{PM} = 0$ and $N_{SM} = N_T$ since there is no driving force for adhesion. For states between these boundary conditions it is reasonable to assume cells would be divided between G_{PM} and G_{SM} according to a Boltzman-like distribution in the form

$$K \equiv N_{PM}/N_{SM} = \exp\{-[\Omega(G_{PM} - G_{SM})]/kT\} - 1 \qquad (3)$$

so that as $G_{PM} \rightarrow G_{SM}$, $N_{PM} \rightarrow 0$, $K \rightarrow 0$; as $G_{PM} \rightarrow -\infty$, $N_{SM} \rightarrow 0$, $K \rightarrow \infty$. The empirical parameter Ω is proportional to cell-substrate contact with cm^2 units. The cell adhesion equilibrium constant K is related to the number of adherent cells N_A (or I_A in % of cell inoculum attached) through $K = N_{PM}/N_{SM} = N_A/N_T - N_A) = I_A/(100 - I_A)$. Since $W_{(sc)}$ is work per unit area it can be identified with the difference $-(G_{PM} - G_{SM})$, leading to the conclusion that $W_{(sc)} = (kt/\Omega)\ (Ln[1 + K])$. Thus, Eq. 3 effectively couples DLVO and wetting theories. Substitution of $W_{(sc)}$ into eq. 2 yields an expression from which the cell wetting tension parameter $\tau^*_{(c)}$ can be calculated from $\tau_{(s)}$ and I_A measurements.

Estimation of Cell Contact Area

Comparison of cell adhesion between different substrates provides a means of estimating values for kT/Ω. Consider evaluation of Eq. 2 for two test substrates designated 1 and 2 for which K and $\tau_{(s)}$ measurements have been made using identical liquid phases. Judicious choice of similar substrates can lead to circumstance that $Ln\ C^0_2 \simeq Ln\ C^0_1$.

Under these conditions, the unknown parameter $\tau^*_{(c)}$ cancels by difference since cells and liquid phases are identical for both substrates. As a means of estimating contact area, it is hypothesized that contact areas on substrates with similar surface chemistry and wetting properties are also similar so that $\Omega_2 \simeq \Omega_1 \equiv \Omega$. Adequacy of this hypothesis will be tested as described in subsequent discussion. Simultaneous solution of Eq. 2 written for substrates 1 and 2 yields an expression for kT/Ω in terms of differences in measured substrate wetting tensions and cell adhesion:

$$kT/\Omega = [\Delta\tau^0_{(s)} - \Delta\tau_{(s)}]/[Ln(1 + K_2) - Ln(1 + K_1)] \quad (4)$$

Subscripted numbers denote substrate 1 or 2 and K values are the cell adhesion equilibrium constants. Determination of contact area for an unrelated *nth* substrate such that $\Omega_n \neq \Omega_1$ and $Ln\ C^0_n \neq Ln\ C^0_1$ cannot be simply determined by difference since $\Delta\tau^*_{(c)} \equiv [\tau^*_{(c,n)} - \tau^*_{(c,1)}] \neq 0$. Fortunately, $\Delta\tau^*_{(c)}$ can be easily evaluated by calculating $\tau^*_{(c,1)}$ at each $X = \text{Ln } C^0_n$ because $\tau^*_{(c,1)} \equiv [\tau_{(c)} - \tau^0_{(c,1)}]|_x = [\tau^0_{(c,n)} - \tau^0_{(c,1)}] = \Delta\tau^0_{(c)}$. Thus, kT/Ω_n can be calculated relative to kT/Ω_1 through Eq. 5:

$$kT/\Omega_n = \{\Delta\tau^0_{(s)} + \Delta\tau^0_{(c)} - \Delta\tau_{(s)} + kT/\Omega_1[Ln(1 + K_1)]\}/Ln(1 + K_n) \quad (5)$$

Eqs. 4 and 5 are valid at any surfactant concentration and can be evaluated at the $Ln\ C$ coinciding with the most sensitive cell adhesion measurements.

Testing the Cell Adhesion Theory

A test of the adequacy of the thermodynamic cell adhesion theory is prediction of qualitative and quantitative aspects of cell adhesion to different substrates and comparison with experimental measurements. A means of performing such a test is estimation of kT/Ω_1 using Eq. 4 and calculation of $\tau^*_{(c,1)}$ as a function of surfactant concentration from Eq. 2. In turn, $\tau^*_{(c,1)}$ can be used to calculate a cell adhesion equilibrium constant for substrates 1 and 2 by rearrangement of Eq. 2. Theoretical values should fit experimental cell adhesion measurements if the approximation that $\Omega_1 \simeq \Omega_2$ is valid. A more general expression for calculation of cell adhesion equilibrium constants K_n applicable to any *nth* substrate unrelated to 1 and 2 can be obtained from Eq. 5 by utilizing values for $\Delta\tau^0_{(c)}$. Thus, theory and experiment can be compared as a test of concepts and approximations uitilized in development of the cell adhesion model. Uncertainty in calculated cell adhesion introduced through experimental error in wetting tension measurements can be estimated by propagation of error through derived equations (see reference 14 for example).

MATERIALS AND METHODS

Materials

Detergent and protein solutions were prepared using Dulbecco's Modified Eagles Medium (DMEM), an isotonic saline solution with added glucose and amino acids widely used in cell culture. Tween-80, a nonionic detergent (polyoxyethylene sorbitan monooleate, molecular weight = 1,309.68, d = 1.064), was used as received from Aldrich Chemical Co., Inc., Milwaukee, WI. Protein solutions were prepared from whole fetal bovine serum (FBS), a protein supplement to culture media. DMEM and FBS were purchased from Gibco Laboratories, Grand Island, NY. Concentrations of surfactant in parts-per-trillion (PPT) are reported in logarithmic units, Ln C. Tissue-culture-grade polystyrene culture dishes (60 × 15 mm, TC PS, parenthesized identification codes that follow correspond to listings in data tables) with surfaces rendered wettable by the manufacturer were purchased from Corning Glass Works, Corning, NY. Bacteriological-grade culture dishes (BG PS) with native, hydrophobic polystyrene surfaces were obtained from Fisher Scientific Co., Allied Corp., Pittsburgh, PA. Polystyrene surfaces with intermediate wettability were prepared by exposing BG PS dishes to 50% chromerge-sulfuric acid solutions (VWR Scientific Div., San Francisco, CA) for 20 min at 37°C (30). Highly wettable surfaces were prepared by exposing BG PS to an oxygen plasma for 5 min (0.5 torr oxygen at 50 W of 13.56 MHz radio-frequency power in a "Plasma Prep" instrument Structure Probe Inc., SPI Supplies Div., West Chester, PA). "Petriperm" (tradename of Heraeus, GMBH), culture dishes with either hydrophilic or hydrophobic "Teflon" FEP film bases (W PP and NW PP, respectively) were purchased from Tekmar Co., Cincinnati, OH. Homemade dishes with polyethylene (PE) film surfaces were prepared as described elsewhere (58).

Cell Culture and Adhesion

Methods employed in the maintenance of cell cultures and determination of equilibrium adhesivity to various substrata were performed as described elsewhere (58). Madin-Darby Canine Kidney cell (MDCK, epithelioid), Rat Basophilic Leukemia (RBL-1), and Human Colon Adenocarcinoma (HCT-15) cells were purchased from American Type Culture Collection, Rockville, Maryland. MDCK and RBL-1 cells were cultured in 10% FBS containing DMEM until needed. HCT-15 was maintained in 20% FBS containing DMEM. MDCK and HCT-15 cells, which grow as an adherent monolayer on tissue-culture flasks, were rinsed twice with Hanks' Balanced Salt Solution (HBSS, without calcium and magnesium) and exposed to trypsin (Gibco, 0.25%) for 5 min. Excess trypsin solution was removed and cells were incubated at 37°C until released from the substrate (~15 min for MDCK and 5 min for HCT-15). Released cells were suspended in DMEM containing the surfactant of choice (FBS or Tween-80) at the concentration of interest. RBL-1 cells had a semi-adherent growth habit. Suspended cells were removed from culture medium by centrifugation and resuspended in DMEM containing the surfactant of choice as in the MDCK case.

Surface Tension and Wetting Measurements

Liquid-vapor (lv) interfacial tensions of surfactant-containing DMEM solutions were measured using an instrumented version of the Whilhelmy plate method (Bimaterials International, Inc., Salt Lake City, Utah). Force-displacement curves exhibited little or no hysteresis (for a general discussion of methods see 22, 29). Contact angles of surfactant-containing liquids on culture-dish surfaces were measured with a contact-angle goniometer (Rame-Hart Inc., Mountain Lakes, NJ). Stable contact angles were obtained within 10 min and substrates were not further characterized by advancing/receding contact angles. Since ordinary plastic materials were utilized in this work, it can be expected that surfaces were heterogeneous on a chemical scale.

Surface-tension curves ($\gamma_{(lv)}$ vs. $Ln\ C$) for surfactant solutions and wetting-tension curves ($\tau_{(s)}$ vs. $Ln\ C$) for test substrates were typically sigmoidal. Consequently, curves were fit to a four-parameter variant of a logistic equation (see, for example, reference 42) which describes a sigmoidal curve and has the form $Y = ([(A - D)/\{1 + (K/Ln\ C)\ \exp N\}] + D)$. The dependent variable Y was either $\gamma_{(lv)}$ for surface-tension curves or $\tau_{(s)}$ for wetting-tension. This procedure provided a simple

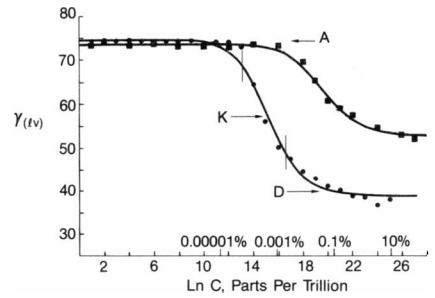


FIGURE 1 Surfactant action of the nonionic detergent Tween-80 (*circles*) and fetal bovine serum (FBS, *squares*). Smooth curves through the data result from fitting to a logistic equation which yields best-fit parameters *A, D,* and *K* (see Materials and Methods section). Surface excess for the Tween case was calculated between the range of concentrations indicated by vertical lines passing through the smooth curve.

method for statistically fitting data and extracting characteristic parameters *A, D, K, N* from $\gamma_{(lv)}$ and $\tau_{(s)}$ curves, yielding smooth functions convenient for computational purposes. The logistic equation has no other special mathematical significance in this application. Physically, *A* and *D* are the low- and high-concentration plateau levels of the sigmoidal curve (see Fig. 1). Consequently, $A_{(lv)}$ corresponds to the interfacial tension of pure DMEM. $A_{(sl)}$ measures DMEM wetting tension on a test substrate with more positive values indicating a more wettable surface. Similarly, $D_{(lv)}$ and $D_{(sl)}$ measure the maximum surfactant effect on $\gamma_{(lv)}$ and $\tau_{(s)}$, respectively. Parameters $K_{(lv)}$ and $K_{(sl)}$ measure surfactant concentration (in Ln *C* units) at half-maximal $\gamma_{(lv)}$ or $\tau_{(s)}$ change, respectively. Surfactants with low critical-micelle concentrations will have characteristically low $K_{(lv)}$ values. The exponential parameter *N* is related to curve slope. Error limits listed in Table I for $A_{(lv)}$, $D_{(lv)}$, $K_{(lv)}$, and $N_{(lv)}$ are standard deviations of six separate $\gamma_{(lv)}$ curve determinations for either Tween-80 of FBS solutions. Mean values are listed for fitted parameters. Error listed for $\tau_{(s)}$ fitted parameters are standard errors of the statistical, least-squares fitting procedure. In all cases reported herein the R-Squared goodness-of-fit parameters was 95% or better. For certain substrates listed in Table I $\tau_{(s)}$ curves were flat. In these cases, wetting tension measurements at various surfactant concentrations were averaged yielding mean and standard deviation values listed under the $A_{(sl)}$ parameter. Quantitative lot-to-lot differences in wetting tension curves for test substrates used as received from the manufacturer were observed during the course of this work. Concentration of surface-active molecules at an interfacial region relative to bulk composition were quantified through use of the Gibbs' relationships (5, 25, 29, 43, 50); $\partial[\gamma_{(lv)}]/\partial[\text{Ln C}] = -RT\Gamma_{(lv)}$, $\partial[\tau_{(s)}]/\partial[Ln\ C] = RT[\Gamma_{(sl)} - \Gamma_{(sv)}]$. Confidence intervals ($1\sigma$) in surface-excess concentrations reported in Table I were estimated from error in fitted coefficients.

RESULTS AND DISCUSSION

Surfactant Action and Adsorption

Fig. 1 compares surfactant action of the nonionic detergent Tween-80 with Fetal Bovine Serum (FBS). As expected for surface-active solutes, $\gamma_{(lv)}$ decreased abruptly as surfactant molecules preferentially adsorbed to tle liquid-vapor interface (25, 43). Surfactant properties of heterogeneous serum-protein mix were similar to that of simple,

TABLE I
SURFACTANT PROPERTIES OF TWEEN-80 AND BOVINE SERUM SOLUTIONS*

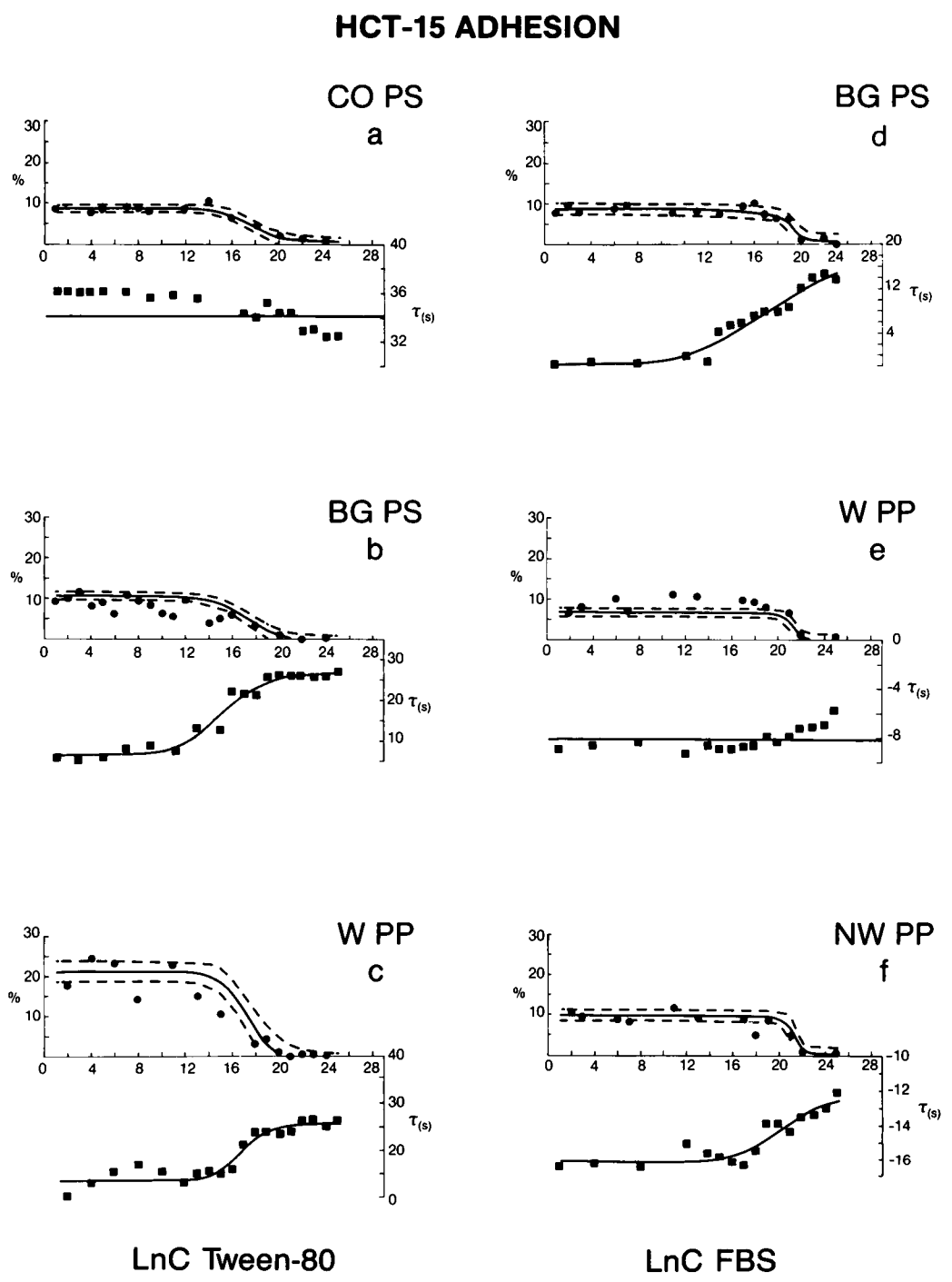
				Liquid-vapor interface‡					Solid-liquid interface§				
				Fitted parameters: $\gamma_{(lv)}$ vs. LnC				Interfacial excess	Fitted parameters: $\tau_{(s)}$ vs. LnC				Interfacial excess
Surfactant				$A_{(lv)}$	$D_{(lv)}$	$K_{(lv)}$	$N_{(lv)}$	$\Gamma_{(lv)}$	$A_{(sl)}$	$D_{(sl)}$	$K_{(sl)}$	$N_{(sl)}$	$\Gamma_{(sl)} - \Gamma_{(sv)}$
(in DMEM)	Cell type	Substrate		dyne/cm	dyne/cm	LnC units		picomoles/cm^2	dyne/cm	dyne/cm	LnC units		picomoles/cm^2
1 Tween-80				74.6 ± 0.5	39.2 ± 1.1	15.9 ± 0.4	−12.0 ± 1.9	237.2 ± 32.4					
2	HCT-15	TC	PS						62.9 ± 0.5	39.4 ± 0.9	15.0 ± 0.2	−15.7 ± 2.2	−217.7 ± 16.2
3		CO	PS						34.1 ± 2.4				
4		W	PS						8.5 ± 0.7	20.6 ± 1.0	16.7 ± 0.5	−15.7 ± 7.1	100.5 ± 17.5
5		BG	PS						6.6 ± 0.8	27.0 ± 1.1	15.1 ± 0.4	−8.4 ± 2.1	101.2 ± 2.6
6 Tween-80	RBL-1	TC	PS						49.7 ± 0.7	36.6 ± 1.2	17.1 ± 0.8	−12.0 ± 5.7	−80.2 ± 14.1
7		CO	PS						30.2 ± 1.7				
8		W	PP						5.0 ± 1.0	17.2 ± 1.1	15.4 ± 0.6	−18.4 ± 12.8	133.2 ± 29.2
9 Tween-80	MDCK	PO	PS						73.6 ± 0.9	38.0 ± 1.0	16.0 ± 0.2	−9.6 ± 1.2	−191.0 ± 13.4
10		TC	PS						60.4 ± 0.5	37.0 ± 0.5	15.6 ± 0.2	−11.8 ± 1.2	−156.9 ± 8.2
11		CO	PS						31.8 ± 3.7				
12		BG	PS						0.0 ± 1.8	26.0 ± 2.0	14.1 ± 0.4	−12.2 ± 4.0	199.8 ± 36.0
13 FBS				74.6 ± 1.6	54.8 ± 1.4	20.5 ± 0.4	−17.2 ± 3.1						
14	HCT-15	BG	PS						−1.7 ± 0.8	20.5 ± 7.5	20.2 ± 3.1	−4.8 ± 1.5	
15		W	PP						−8.1 ± 0.9				
16		NW	PP						−16.2 ± 0.2	−12.0 ± 1.1	20.6 ± 1.5	−9.3 ± 5.4	
17 FBS	RBL-1	BG	PS						0.6 ± 3.3				
18		W	PP						−5.4 ± 2.0				
19		NW	PP						−11.8 ± 2.4				
20 FBS	MDCK	BG	PS						4.0 ± 0.8	12.3 ± 1.3	22.2 ± 0.5	−39.9 ± 17.9	
21		PE							−4.0 ± 0.7	4.5 ± 2.8	22.8 ± 1.5	−16.0 ± 12.6	
22		NW	PP						−22.7 ± 1.5	−7.6 ± 4.1	22.1 ± 1.7	−7.0 ± 4.3	

*Abbreviations: DMEM = Dulbecco's Modified Eagle Medium; TC PS = tissue culture grade polystyrene; CO PS = chromerge oxidized polystyrene; W PP = wettable Petriperm®; BG PS = bacteriological grade polystyrene; PO PS = plasma oxidized polystyrene; FBS = fetal bovine serum; PE = polyethylene; NW PP = nonwettable Petriperm®. See Methods and Materials section for details on substrates and cells.

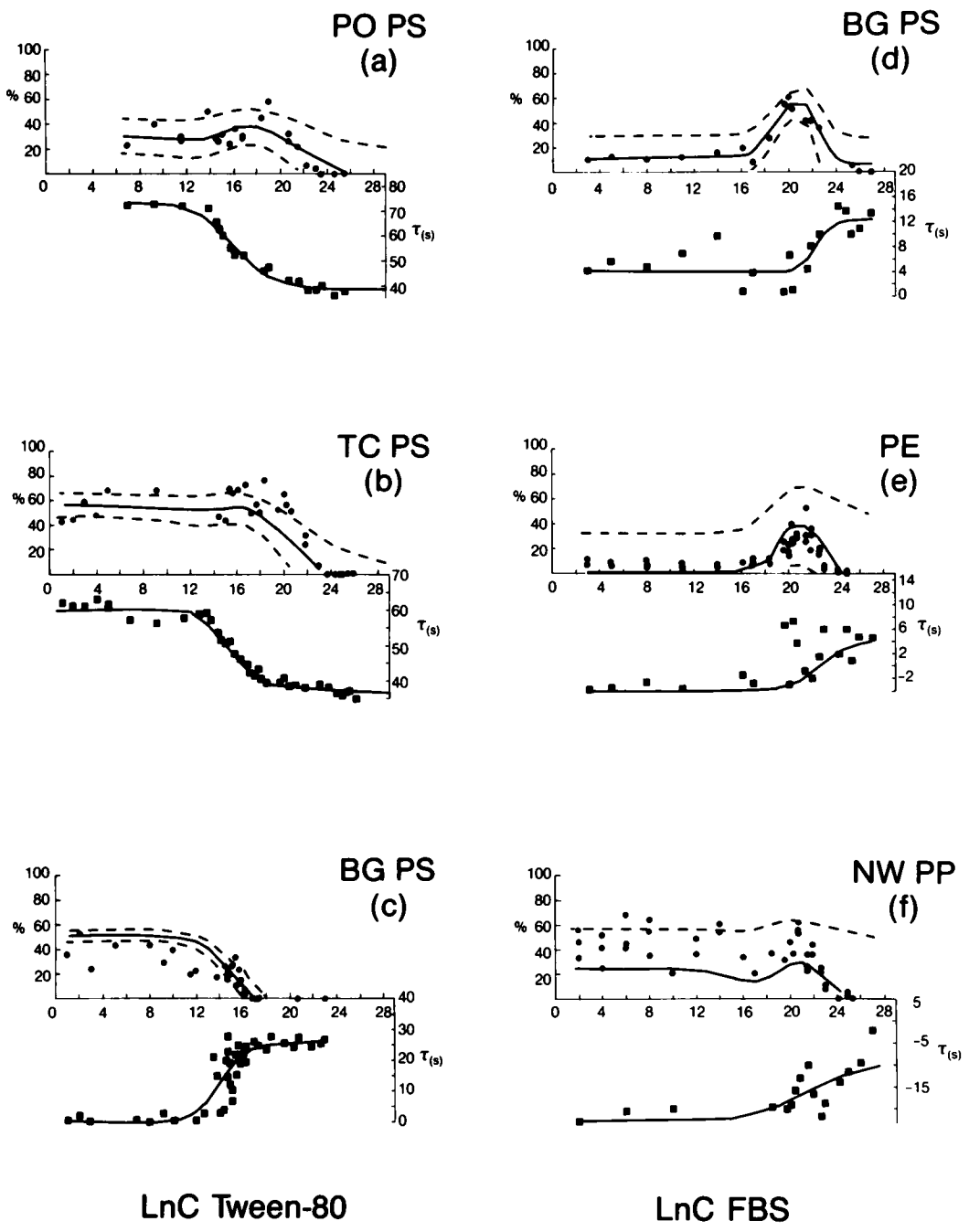
‡Average fitted parameters ± standard deviations for six trials ($n = 6$) for Tween-80 and FBS solutions in DMEM.

§See Materials and Methods section for discussion of error estimates.

binary detergent solution but FBS was a much weaker surfactant system. Smooth curves through the data of Fig. 1 result from least-squares fitting to a logistic equation (as described in the Materials and Methods section) which yielded the characteristic parameters $A_{(lv)}$, $D_{(lv)}$, $K_{(lv)}$, and $N_{(lv)}$ collected in Table I. Surface excess $\Gamma_{(lv)}$ of Tween-80 (for which density and molecular weight are known) was calculated between the range indicated by vertical lines intersecting the $\gamma_{(lv)}$ curve. Average $\Gamma_{(lv)}$ listed in Table I is in reasonable agreement with expected values (43), indi-



FIGURES 2 and 3 *a–f* Equilibrium cell adhesion to various plastic substrates as a function of surfactant concentration (*left-hand, upper axis; circles* in % inoculum attached; *a–c* correspond to *adhesion from Tween-80 solutions; d–f correspond to* adhesion from FBS solutions). Smooth curves through the data were calculated from the thermodynamic theory of cell adhesion. Dashed lines bounding smooth curves represent uncertainty in theoretical adhesion. Right-hand, lower axis plots substrate wetting tension $\tau_{(s)}$ (*squares* in dynes/cm). Cell identity is given in figure titles. Substrate identity codes are given at the upper right corner of *a–f* (see Materials and Methods section for substrate and cell description).



FIGURES 2 and 3 (Continued)

cating a concentration in excess of bulk composition of 237.2 picomoles Tween per unit area of interface.

The driving force for solute partitioning is interaction of solvent molecules which tends to expel hydrophobic portions of surfactants from solution to the nonpolar vapor interface (29). These same solvent-molecule interactions are also responsible for surfactant adsorption to the (sl) interface. In this case, adsorption is detected and quantified by measurement of wetting tensions, $\tau_{(s)}$, which is the result of a mechanical equilibrium between (sv), (sl), and (lv) interfacial tensions. Adsorption of surface-active solutes at these interfaces affects this equilibrium causing measurable changes in $\tau_{(s)}$. Figs. 2 *a–f* and 3 *a–f* collect $\tau_{(s)}$ curves for various plastic substrates studied in this work (right-hand lower axes, squares; results for RBL-1 cells not shown). Smooth curves through data were obtained by fitting to the logistic equation, as described above for the $\gamma_{(lv)}$ case. Characteristic fitted parameters $A_{(sl)}$, $D_{(sl)}$, $K_{(sl)}$,

and $N_{(sl)}$ are collected in Table I along with calculated surface excess $[\Gamma_{(sl)} - \Gamma_{(sv)}]$. Substrates have been ordered in decreasing DMEM wettability [decreasing $A_{(sl)}$].

For illustration of results, compare $\tau_{(s)}$ curves for oxygen-plasma etched polystyrene (PO PS, Fig. 3 *a*) and bacteriological-grade polystyrene (BG PS, Fig. 3 *c*). Plasma oxidation rendered polystyrene surfaces highly wettable with $A_{(sl)} = 73.6$ and undoubtedly highly ionic in character (4, 19, 30, 41, 49). BG PS was in the native, nonwettable state yielding $A_{(sl)} = 0.0$. In the PO PS case, $[\Gamma_{(sl)} - \Gamma_{(sv)}] = -191.0$ (negative $\tau_{(s)}$ curve slope) indicating that the (sl) interface was depleted in surfactant relative to the (sv) interface. By contrast $[\Gamma_{(sl)} - \Gamma_{(sv)}] = 199.8$ (positive slope) for BG PS demonstrating a concentration of Tween at the (sl) interface. Apparently, Tween-80 was expelled from solution and adsorbed to the hydrophobic BG PS interface whereas water molecules strongly associated with polar moieties on PO PS, causing Tween to be less concentrated at this interface relative to bulk composition. Similarly, FBS proteins adsorb to BG PS (Fig. 3 *d*) as expected for a hydrophobic surface.

Cell Adhesion Measurements and Parameters

Equilibrium cell adhesion to plastic substrates listed in Table I at various surfactant concentrations are shown in Figs. 2–3 (left-hand upper axes, circles, in percent of inoculum attached). Adhesion curves (I_A vs. *Ln C*) can be generally classified by shape; plateau (Figs. 2 *a–f*, 3 *c*), peak (Figs. 3 *d–e*), or peak-plateau (3 *a–b, f*) profile. RBL-1 adhesion curves (not shown) were plateau type for both Tween-80 and FBS solutions as in the HCT-15 case. For each combination of cell type and substrate composition, adhesion could be driven to zero at empirically determined surfactant compositions *Ln* C^0, as reported in Table II. Substrates have been ordered in decreasing DMEM wettability to correspond with Table I. Substrate number 1 or 2 differentiates standard and reference surfaces, respectively. Adhesion to substrates 1 and 2 were compared with each cell type and values for kT/Ω estimated according to computational procedures outlined in the Theory section. Generally, for each cell type studied, *Ln* C^0 and Ω values decreased with decreasing substrate wetting tension. Within a particular cell type, contact area was greatest on surfaces treated by ionizing corona or plasma discharges to increase wettability (PO PS, TC PS, W PP). Relative to the dimensions of a cell (10–20 μm diameter), estimated Ω values were very small. However, it has been shown that cells make initial substrate contacts using microvilli with dimensions on the order 0.1 μm (16, 29). The magnitude of calculated areas is most consistent with an adhesion model based on substrate interaction with a "fuzzy," polysaccaride-coated cell membrane (39) and divalent ion bridging.

Interpretation of the contact-area parameter must be made cautiously, particularly when making comparisons between cell types and liquid phases, since proportionality between Ω and actual adhesion area is unknown. Nevertheless, it is of interest to compare adhesion with a particular substrate from the same liquid phase for the different cell types studied. From Table II, with respect to adhesion to TC PS from Tween-80 solutions, it can be seen that Ω values for the continuous, adherent cell line MDCK were greater than that of either the leukemic blood cell RBL-1 or the anaplastic HCT-15 cell line. This is consistent with the weakly adherent growth habit observed for these cells and the general consensus that neoplastic cells are poorly adherent to tissue-culture polystyrene (11, 27, 40). Contact area calculated for cell adhesion from FBS solutions was greater for two of the three cell types. For example, compare MDCK adhesion with BG PS and RBL-1 to W PP from Tween and FBS solutions. Increased contact area is consistent with observed partial spreading of cells in FBS relative to Tween solutions during the course of attachment measurements and to putative adhesion factors present in serum which aid cellular adherence (27). Contact area for HCT-15 was consistently low and independent of surfactant type.

Cell Wetting Tension

Fig. 4 collects $\tau^*_{(c)}$ values as a function of surfactant concentration calculated from Eq. 2 for the different cell lines studied. Smooth lines through the data result from least-squares fitting to the logistic equation which yielded characteristic parameters $A_{(cl)}$, $D_{(cl)}$, $K_{(cl)}$, and $N_{(cl)}$ reported in Table II. Although cell wetting parameters were deduced from adhesion measurements through a somewhat convoluted theoretical process, $\tau^*_{(c)}$ curves can be interpreted in a manner analogous to the more conventionally-determined $\tau_{(s)}$ curves. That is, a positive $\tau^*_{(c)}$ curve slope indicates adsorption of surfactant at the (cl) interface. A flat slope indicates no preferential surfactant partitioning from bulk solution. From Fig. 4 it can be deduced that Tween adsorbs to membranes of all three cell types investigated. By contrast, FBS proteins were only weakly adsorbed to HCT-15 cells and not preferentially adsorbed to MDCK and RBL-1 relative to bulk composition. Quantitative values for $[\Gamma_{(cl)} - \Gamma_{(cv)}]$ for the Tween case are listed in Table II.

Theoretical Equilibrium Cell Adhesion

Solid lines drawn through experimentally-measured cell adhesion shown in Figs. 2–3 were calculated from the thermodynamic theory of cell adhesion. Dashed lines bounding smooth curves represent uncertainty in theoretical cell adhesion introduced by error in wetting tension measurements. Theory and experiment were in semiquantitative agreement for adhesion to all substrates from both Tween-80 and FBS solutions (note changes in % adherence scales within Figures). Error in wetting measurements for cell and substrates translates into an amplified uncertainty in calculated adhesion equilibrium constants through an

TABLE II
CELL ADHESION AND MEMBRANE WETTING PROPERTIES*

							Calculated cell parameters‡				
							Fitted parameters: $\tau_{(c)}$ vs. LnC				Interfacial excess
Surfactant			Substrate		LnC°	Ω	$A_{(c1)}$	$D_{(c1)}$	$K_{(c1)}$	$N_{(c1)}$	$\Gamma_{(c1)} - \Gamma_{(cv)}$
(in DMEM)	Cell type	Substrate	Number		LnC units	$cm^2 \times 10^{+16}$					picomoles/cm^2
1 Tween-80	HCT-15	TC PS			22.8	1.9	−91.6 ± 3.5	−3.8 ± 8.2	17.5 ± 0.6	−13.8 ± 5.5	629.1 ± 117.6
2		CO PS	1		22.0	0.4					
3		W PP			20.1	1.2					
4		BG PS	2		21.6	0.5					
5 Tween-80	RBL-1	TC PS			25.0	14.7					
6		CO PS	2		23.9	2.1					
7		W PP	1		16.9	1.9	−28.1 ± 1.6	−4.3 ± 1.7	13.5 ± 0.4	−24.5 ± 16.6	403.0 ± 68.8
8 Tween-80	MDCK	PO PS	1		23.9	16.2	−44.5 ± 2.3	4.8 ± 4.2	18.2 ± 0.7	−6.4 ± 1.2	154.2 ± 25.1
9		TC PS	2		23.6	19.4					
10		CO PP			19.2	7.2					
11		BG PS			16.9	7.2					
12 FBS	HCT-15	BG PS	1		24.6	0.7	−42.8 ± 2.3	−4.8 ± 5.8	21.2 ± 0.4	−69.9 ± 28.4	
13		W PP	2		24.0	0.8					
14		NW PP			23.7	1.1					
15 FBS	RBL-1	BG PS	2		24.1	5.5					
16		W PP			25.7	21.3					
17		NW PP	1		24.0	4.5	−8.1 ± 0.3	−1.4 ± 0.5	20.0 ± 0.2	−60.8 ± 34.1	
18 FBS	MDCK	BG PS	1		26.5	50.5	7.2 ± 0.5	−0.6 ± 0.6	19.2 ± 0.4	−20.0 ± 7.3	
19		PE	2		24.5	58.1					
20		NW PP			25.0	45.5					

*Abbreviations: DMEM = Dulbecco's Modified Eagle Medium; TC PS = tissue culture grade polystyrene; CO PS = chromerge oxidized polystyrene; W PP = wettable Petriperm®; BG PS = bacteriological grade polystyrene; PO PS = plasma oxidized polystyrene; FBS = fetal bovine serum; NW PP = nonwettable Petriperm®. See Methods and Materials section for details on substrates and cells.
‡See Materials and Methods section for discussion of error estimates.

additive effect. Estimated contact area enters into error calculation as an exponential factor. Consequently, uncertainty in theoretical adhesion was greatest for poorly-characterized substrates and cells with larger contact area (MDCK adhesion from FBS solutions for example). Variability in $\tau_{(s)}$ curves was ultimately traced to plate-to-plate differences within a lot of culture dishes. Precision of this work could be improved by using standardized substrate materials with uniform and chemically homogeneous surfaces.

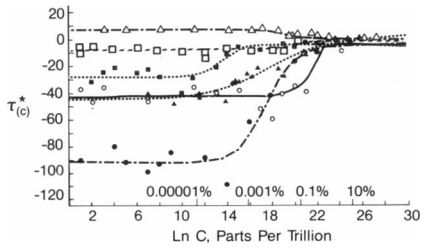


FIGURE 4 Cell wetting-parameters $\tau^*_{(c)}$ as a function of surfactant concentration (*filled symbols* correspond to Tween-80 solutions, *empty symbols* correspond to FBS solutions; *circles* = HCT-15, *squares* = RBL-1; *triangle* = MDCK).

Agreement between theory and experiment is taken as corroboration of the proposal that the plateau in cell attachment-rate curves is a result of thermodynamic equilibrium (58) and strong support for the concepts that (*a*) the Dupré equation recast in terms of cell and substrate wetting tensions can be applied to cell adhesion measurements; (*b*) cell attachment from sessile liquid phases can be described by a DLVO-like theory of attractive forces and repulsive barriers; (*c*) cell-adhesion equlibrium constants are related to $W_{(sc)}$ through a free energy relationship. Furthermore, assumptions that $\gamma_{(sc)}$, $\gamma_{(sv)}$, $\gamma_{(cv)}$, and contact area Ω are not strong functions of liquid-phase composition were apparently reasonable approximations.

CONCLUSIONS

Equilibrium cell adhesion is controlled by an arithmetic combination of substrate and cell wetting tensions. Wetting tensions, in turn, are controlled by adsorption of soluble surfactants to cell-liquid and substrate-liquid interfaces. Effect of serum proteins on short-term cell adhesion (<2 h) is that of a biosurfactant, similar in action to the ordinary detergent Tween-80. Of course, nutritive and mitogenic factors present in serum will dramatically effect

long-term adhesion, cell growth, and the ultimate expression of a biological component to work of adhesion. But the physicochemically-based DLVO theory of colloid science is an adequate basis to explain qualitative and quantitative aspects of short-term cellular adherence from a sessile liquid phase. In conjunction with a modified thermodynamic description of adhesion which treats biological cells as ordinary physical objects, parameters related cell wetting tension and cell-substrate contact area can be calculated for use both as a diagnostic and predictive tool in biomaterial science.

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REFERENCES

1. Absolom, D. R., C. Thomson, G. Kruzyk, W. Zingg, and A. W. Neumann. 1986. Adhesion of hydrophilic particles (human neutrophils) to polymer surfaces: effects of pH and ionic strength. *Colloid Surf.* 21:447–456.
2. Absolom, D. R., C. J. van Oss, R. J. Genco, D. W. Francis, and A. W. Neumann. 1980. Surface thermodynamics of normal and pathological granulocytes. *Cell Biophys.* 2:113–126.
3. Adamson, A. W. 1967. *In* Physical Chemistry of Surfaces. Interscience, Publishers, New York. 465–467.
4. Amstein C. F., and P. A. Hartman. 1975. Adaptation of plastic surfaces for tissue culture by glow discharge. *J. Clin. Microbiol.* 2:46–54.
5. Aveyard, R., and D. Haydon. 1973. *In* An Introduction to the Principles of Surface Chemistry. Cambridge University Press, London. 195–223.
6. Baier, R. E. 1986. Modification of surfaces to meet bioadhesive design goals: a review. *J. Adhesion.* 20:171–186.
7. Baier, R. E. 1985. Adhesion in the biologic environment. Biomater. Med. *Devices Artif. Organs.* 12:133–159.
8. Baier, R. E. 1977. The organization of blood components near interfaces. *Ann. NY Acad. Sci.* 283:17–36.
9. Baier, R. E. 1976. Initial events at the blood/biomaterial border. *In* Procs. 8th Sci. Conf. of Gesell. Deutscher Natire und Alryte. 13:159–162.
10. Baier, R. E. 1972. The role of surface energy in thrombogenesis. *Bull. NY Acad. Med.* 48:257–272.
11. Banerjee, D., R. J. W. Pentney, I. Chackalalparampil, and B. B. Mukherjee. 1986. Ability of oncogenically transformed cells to grow without anchorage correlates with phosphorylation of a group cell surface membrane proteins. *Exp. Cell. Res.* 166:442–454.
12. Barngrover, D. 1986. Substrata for anchorage-dependent cells. *In* Mammalian Cell Technology. W. G. Thilly, editor. Butterworths Publishers, Boston, MA. 131–149.
13. Berger, K., L. R. Sauvage, A. M. Rao, and S. J. Wood. 1972. Healing of arterial prostheses in man: its incompleteness. *Ann. Surg.* 175:118–127.
14. Bevington, P. R. 1969. *In* Data Reduction and Error Analysis for the Physical Sciences. McGraw-Hill Inc., New York. 56–65.
15. Benedict, R. W., and M. C. Williams. 1979. Bonding erythrocytes to plastic substrates by glow-discharge activation. *Biomat. Med. Devices Artif. Organ.* 7:477–493.
16. Bongrand, P., C. Capo, and R. Depieds. 1982. Physics of cell adhesion. *Prog. Surf. Membr. Sci.* 12:217–286.
17. Busscher, H. J., A. H. Weerkamp, C. C. van der Mei, A. W. J. van Pelt, H. P. de Jong, and J. Arends. 1984. Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. *Appl. Environ. Microbiol.* 48:980–983.
18. Chu, C. C., and Williams, D. F. 1984. Effects of physical configuration and chemical structure of suture materials on bacterial adhesion. *Am. J. Surg.* 147:197–204.
19. Curtis, A. S. G., Forrester, J. V., C. McInnes, and F. Lawrie. 1983. Adhesion of cells to polystyrene surfaces. 1983. *J. Cell. Biol.* 97:1500–1506.
20. Dahlgren, C., H. Elwing, and K. E. Magnusson. 1986. Comparison of contact angles calculated from the diameter of sessile drops and submerged air bubbles in contact with a solid surface. *Colloids Surf.* 17:295–303.
21. Dankert, J., A. H. Hogt, and J. Feijen. 1986. Biomedical polymers: bacterial adhesion, colonization, and infection. *CRC Crit. Rev. Biocompat.* 2:219–301.
22. Gerson, D. F. 1981. Methods in surface physics for immunology. *Immunol. Meth.* 2:105–137.
23. Gerson, D. F. 1981. Interfacial free energies of cells and polymers in aqueous media. *In* Physicochemical Aspects of Polymer Surfaces. 1:229–240.
24. Gerson, D. F. 1980. Cell surface energy, contact angles and phase partition. I. lymphocytic cell lines in biphasic aqueous mixtures. *Biochim. Biophys. Acta.* 602:269–280.
25. Gerson, D. F., and J. E. Zajic. 1979. Microbial Biosurfactants. *Process Biochem.* 14:20–29.
26. Gingell, D., and S. Vince. 1980. Long-range forces and adhesion: an analysis of cell-substratum studies. *In* Cell Adhesion and Motility, Third Symposium of the British Society for Cell Biology. A. S. G. Curtis and J. D. Pitts, editors. Cambridge University Press, London. 1–38.
27. Grinnell, F. 1978. Cellular adhesiveness and extracellular substrata. *Int. Rev. Cytol.* 53:65–144.
28. Gristina, A. G., M. Oga, L. X. Webb, and C. D. Hobgood. 1985. Adherent bacterial colonization in the pathogenesis of osteomyelitis. *Science (Wash. DC).* 228:990–993.
29. Johnson, R. E., and R. H. Dettre. 1969. Wettability and contact angles. *Surf. Colloid Sci.* 2:85–153.
30. Klemperer, H. G., and P. Knox. 1977. Attachment and growth of BHK cells and liver cells on polystyrene: effect of surface groups introduced by treatment with chromic acid. *Lab. Pract.* 26:179–180.
31. Marmur, A., and E. Ruckenstein. 1986. Gravity and Cell Adhesion. *J. Colloid Interface Sci.* 114:261–266.
32. Mittal, K. L. 1975. Surface chemical criteria for maximal adhesion and their verification against the experimentally measured adhesive strength values. *Adhesion Sci. Technol.* 9A:129–168.
33. Muggli, R., and H. R. Baumgartner. 1978. Platelet interactions with collageneous substrates in the presence of flowing blood. *Suppl. Thromb. Haemostas.* 63:289–300.
34. Neumann, A. W., O. S. Hum, D. W. Francis, W. Zingg, and C. J. van Oss. 1980. Kinetic and thermodynamic aspects of platelet adhesion from suspension to various substrates. *J. Biomed. Mater. Res.* 14:499–509.
35. Neumann, A. W., D. R. Absolom, C. J. van Oss, and W. Zingg. 1979. Surface thermodynamics of leukocyte and platelet adhesion to polymer surfaces. *Cell* Biophys. 1:79–92.
36. Neumann, A. W., C. J. Hope, C. A. Ward, M. A. Herbert, G. W. Dunn, and W. Zingg. 1975. The role of surface thermodynamics in thromboresistance of biomaterials. *J. Biomed. Mater. Res.* 9:127–142.
37. Neumann, A. W., R. J. Good, C. J. Hope, and M. Sejpal. 1974. An equation-of-state approach to determine surface tensions of low-energy solids from contact angles. *J. Colloid. Interface Sci.* 49:291–304.
38. Olsen, D. A., and H. D. Kletschka. 1973. Biophysical interactions of

blood proteins with polymeric and artificial surfaces. 1973. *Prog. Surf. Membr. Sci.* 6:332–359.
39. Parsegian, V. A., and D. Gingell. 1973. A physical force model of biological membrane interactions. *In* Recent Advances in Adhesion. L. H. Lee, editor. Gordon and Breach, Science Publishers Inc., New York. 153–192.
40. Pethica, B. A. 1983. Microbial and cell adhesion. In Microbial Adhesion to Surfaces. R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, editors. Ellis Horwood Ltd., Chichester. 16–45.
41. Ramsey, W. S., W. Hertl, E. D. Nowlan, and N. J. Binkowski. 1984. Surface treatments and cell attachment. *In Vitro Cell Dev. Bio.* 20:802–808.
42. Rodbard, D. 1981. Mathematics and statistics of ligand assays: an illustrated guide. *In* Ligand Assay. J. Langan and J. J. Clapp, Editors. Masson Publishing, New York. 45–101.
43. Rosen, M. J. 1978. Adsorption of surface-active agents at interfaces. *In* Surfactants and Interfacial Phenomena. John Wiley & Sons Inc., New York. 25–82.
44. Rutter, P. R., and B. Vincent. 1983. The adhesion of microorganisms to surfaces: physico-chemical aspects. *In* Microbial Adhesion to Surfaces. R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, editors. Ellis Horwood Ltd., Chichester. 16–45.
45. Rutter, P. R. 1980. The physical chemistry of the adhesion of bacteria and other cells. *In* Cell Adhesion and Motility. Third Symposium of the British Society for Cell Biology. A. S. G. Curtis and J. D. Pitts, editors. Cambridge University Press, London. 103–135.
46. Schmitt, D. D., D. F. Bandyk, A. J. Pequet, and J. B. Towne. 1986. Bacterial adherence to vascular prostheses. *J. Vasc. Surg.* 3:732–740.
47. Schurch, S., D. F. Gerson, and D. J. L. McIver. 1981. Determination of cell/medium interfacial tensions in aqueous polymer systems. *Biochim. Biophys. Acta.* 640:557–571.
48. Seitz, T. L., K. D. Noonan, L. L. Hench, N. E. Noonan. 1982. Effect of fibronectin on the adhesion of an established cell line to a surface reactive biomaterial. *J. Biomed. Mater. Res.* 16:195–207.
49. Smith, L., D. Hill, J. Hibbs, S. W. Kim, J. Andrade, and D. Lyman. 1975. Glow discharge surface treatment for improved cellular adhesion. *Polymer Prepr.* 16:186–190.
50. Smolders, C. A. 1967. Wetting of polymers-effect of surfactant adsorption. *Proc. Int. Cong. Surf. Active Agents* 2:343–349.
51. Spence, M. R. 1984. Infections associated with intrauterine contraceptive devices. *In* Infections Associated With Prosthetic Devices. B. Sugerman and E. J. Young Editors. CRC Press, Boca Rattan, Fl. 243–250.
52. Srinivasan, R., and E. Ruckenstein. 1981. Kinetically caused saturation in the deposition of particles or cells. *J. Colloid Interface Sci.* 79:390–398.
53. Tenney, J. H., M. R. Moody, K. A. Newman, S. C. Schimpff, J. C. Wade, J. W. Costerron, and W. P. Reed. 1986. Adherent microorganism on lumenal surfaces of long-term intravenous catheters. *Arch. Intern. Med.* 146:1949–1954.
54. Torney, D. C., M. Dembo, and G. I. Bell. 1986. Thermodynamics of cell adhesion. II. *Biophys. J.* 49:501–507.
55. Uyen, M., H. J. Busscher, A. H. Weerkamp, and J. Arends. 1985. Surface free energies of oral streptococci and their adhesion to solids. *FEMS (Fed. Eur. Microbiol. Soc.) Lett.* 30:103–106.
56. Van der Valk, P., A. W. J. van Pelt, H. J. Busscher, H. P. de Jong, R. H. Wildevuur, and J. Arends. 1983. Interaction of fibroblasts and polymer surfaces: relationship between surface free energy and fibroblast spreading. *J. Biomed. Mater. Res.* 17:807–817.
57. Van Oss, C. J., C. F. Gillman, and A. W. Neumann. 1975. *In* Phagocytic Engulfment and Cell Adhesiveness. Marcel Dekker Inc., New York. 84–99.
58. Vogler, E. A., and R. W. Bussian. 1987. Short-term cell-attachment rates: a surface-sensitive test of cell-substrate compatibility. *J. Biomed. Mater. Res.* 21:1197–1211.
59. Vroman, L. 1981. What factors determine thrombogenicity. *Bull. N.Y. Acad. Med.* 48:302–309.
60. Ward, C. A., and A. W. Neumann. 1974. On the surface thermodynamics of a two-component liquid-vapor-ideal solid system. *J. Colloid Interface Sci.* 49:286–290.
61. Weiss, L., and J. P. Harlos. 1972. Some speculations on the rate of adhesion of cells to coverslips. *J. Theor. Biol.* 37:169–179.