TOPICAL REVIEW

Soft particle analysis of electrokinetics of biological cells and their model systems

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Abstract
In this article, we review the applications of a novel theory (Ohshima 2009 Sci. Technol. Adv. Mater. 10 063001) to the analysis of electrokinetic data for various soft particles, that is, particles covered with an ion-permeable surface layer of polyelectrolytes. Soft particles discussed in this review include various biological cells and hydrogel-coated particles as a model of biological cells. Cellular transformations increase the concentration of sialic acid of glycoproteins and are associated with blocked biosynthesis of glycolipids and aberrant expression of the developmentally programmed biosynthetic pathway. The change in shape or biological function of cells may affect their surface properties and can be detected by electrokinetic measurements. The experimental results were analyzed with Ohshima’s electrokinetic formula for soft particles and soft surfaces. As a model system, hydrogel surfaces that mimic biological surfaces were also prepared and their surface properties were studied.

Keywords: electrokinetics, soft particles, soft surfaces, hydrogel, biological cells

1. Introduction

Biological cells usually have surfaces coated with an ion-permeable surface layer of polyelectrolytes. Such particles and their surfaces are often referred to as soft particles and soft surfaces, respectively. Their behavior differs from that of conventional colloidal particles without structured surfaces, which are called hard particles. Generally, the electrokinetic data (e.g. electrophoretic mobility and electro-osmotic velocity) of hard particles are converted to their zeta potentials. For soft particles, however, the concept of zeta potential loses its physical meaning and a different mobility formula is required. Ohshima [1–4] has developed a novel electrokinetic theory of soft particles, the theory was applied to the study of the electrokinetics of various soft particles. Here, we apply that theory to the analysis of electrokinetic data for various soft particles and soft surfaces including latex particles covered with temperature-sensitive hydrogel layers, HL-60 RG cells, RAW 117-P cells, rat leukemia cells and PLGA microspheres.

Biological cell membranes are soft and have soft polymer layers composed of organic molecules, such as sugar moieties, on their surfaces [5–10]. Some hydrogels change their structures and properties in response to external stimuli, such as temperature, pressure and pH [11]. Their stimulus-sensitive structural changes are accompanied by structural changes in their surface layers. We have measured the electrophoretic mobility of soft particles and the electroosmotic flow on
soft surfaces and analyzed the surface properties using Ohshima’s electrokinetic formula for soft particles and soft surfaces [1–4].

2. Electrokinetic formula for soft particles and soft surfaces

To evaluate the structural difference between surface layers, we analyze the electrohydrodynamic mobility data with the soft particle model proposed by Ohshima [1–4]. This model considers a solid sphere coated with an ion-permeable layer of polyelectrolytes or charged polymers moving with a velocity $U$ in an external electric field $E$. The electrohydrodynamic mobility $\mu$ is calculated as $\mu = U/E$, where $U = |U|$ and $E = |E|$. We regard the polymer segments as resistance centers and assume that they are distributed uniformly in the polyelectrolyte layer, exerting frictional forces on the liquid flowing in the polyelectrolyte layer. Figure 1 schematically presents the ion distribution, potential distribution $\psi(x)$ and liquid velocity distribution $u(x)$ around a soft sphere. The sphere consists of a hard particle core of radius $a$ coated with an ion-permeable surface layer of polyelectrolytes of thickness $b-a$, where $x$ is the distance measured from the particle core.

The potential deep inside the surface layer is close to the Donnan potential. Thus, we define the surface potential of a soft particle at the outer edge of the surface layer. We solve the Navier–Stokes equations outside and inside the surface charge layer by introducing the frictional term due to the polymer segments into the Navier–Stokes equation for the internal region of the surface layer. We finally obtain the following expression for the electrophoretic mobility of a soft particle [1–4]:

$$\mu = \frac{e_0 \psi_o / \kappa_m + \psi_{DON}/\lambda}{1/\kappa_m + 1/\lambda} + \frac{zeN}{\eta \lambda^2},$$

with

$$\psi_{DON} = \frac{kT}{ve} \ln \left[ \frac{zN}{2vn} + \left( \frac{zN}{2vn} \right)^2 + 1 \right]^{1/2},$$

$$\psi_o = \frac{kT}{ve} \left( \ln \left[ \frac{zN}{2vn} + \left( \frac{zN}{2vn} \right)^2 + 1 \right]^{1/2},$$

$$\frac{2vn}{zN} \left[ 1 - \left( \frac{zN}{2vn} \right)^2 + 1 \right]^{1/2},$$

$$\lambda = (\gamma/\eta)^{1/2},$$

$$\kappa_m = \kappa \left[ 1 + \left( \frac{zN}{2vn} \right)^2 \right]^{1/4},$$

$$\kappa = \left( \frac{2ne^2 \psi^2}{e_0 \kappa T} \right)^{1/2}.$$
3. Electrophoretic mobility of latex particles covered with temperature-sensitive hydrogel layers

In this section, we present the electrokinetic analysis of poly(N-isopropylacrylamide) (poly-NIPAM) hydrogel-coated latex particles, which are good examples of soft particles, since they are coated with a polyelectrolyte. Poly-NIPAM hydrogel is temperature-sensitive and exhibits a phase transition at 33°C [12–14], i.e. it swells below 33°C and shrinks above 33°C. Monodisperse latex particles composed of a poly(styrene-co-ethylene glycol methacrylate) core and a poly-NIPAM hydrogel surface layer were prepared using a soap-free emulsification method. The latex particles show a marked change in their diameter at 33°C, which is attributed to the change in the thickness of the poly-NIPAM hydrogel layer.

The electrophoretic mobility of the latex particles was measured in an electrolyte solution as a function of the ionic strength and temperature of the suspending media [15, 16]. The latex particles were dispersed in phosphate buffer solutions (pH 7.4) having different ionic strengths at four different temperatures: 25, 30, 35 and 40°C. Experimental results in figure 2 reveal marked differences between the mobility values above and below the phase transition temperatures of poly-NIPAM, which correlate well with the size of latex particles.

Equation (1) contains two unknown parameters, \( zN \) and \( 1/\lambda \), which were obtained through curve fitting. The fitting curves agree well with the experimental results obtained over a wide range of the ionic strength values, as shown in figure 2. The deviation at low ionic strengths is caused by the hydrogel layer undergoing an additional minor expansion due to the reduction in the extent of ionic shielding that decreases \( N \). The overall agreement between the theoretical and experimental results implies that hydrogel-layer-coated latex particles show the electrophoretic behavior of ‘soft particles’ described by equation (1). This allows the estimation of \( N \) and \( 1/\lambda \) via curve fitting. The best fit (solid lines in figure 2) is achieved with \( zN = -0.0015 \) M and \( 1/\lambda = 1.2 \) nm at 25°C; \( zN = -0.0025 \) M and \( 1/\lambda = 1.2 \) nm at 30°C; \( zN = -0.03 \) M and \( 1/\lambda = 0.9 \) nm at 35°C or 40°C.

The observed small difference in mobility between 35 and 40°C can be explained by the temperature dependence of the relative permittivity \( \varepsilon_r \) and the viscosity \( \eta \) of the suspending media. This implies that \( zN \) and \( 1/\lambda \) do not change appreciably between 35 and 40°C. On the other hand, the large mobility increase from 30 to 35°C across the phase transition temperature mainly originates from an increase in the charge density \( zN \) with a slight decrease in \( 1/\lambda \). That is, below the phase transition temperature, where the hydrogel is swollen, the charge density is low, while above the phase transition temperature, where the hydrogel is shrunken, the charge density is high. These variations result in a large change in mobility.

The minor differences in the estimated \( zN \) between 25 and 30°C is also due to a small change in the thickness of the hydrogel layer in this temperature range. \( 1/\lambda \) decreases as the drag exerted by the hydrogel layer on the liquid flow increases. In the limit \( 1/\lambda \to 0 \) equation (1) approximates the well-known Smoluchowski’s mobility formula for ‘hard particles’. In other words, as \( 1/\lambda \) increases, the hydrogel layer on the particle becomes ‘softer’, that is, \( 1/\lambda \) can be used to characterize the ‘softness’ of the hydrogel layer on the particle. The observed decrease in \( 1/\lambda \) from 30 to 35°C implies that the hydrogel layer becomes ‘harder’, in accordance with the observed shrinkage of the hydrogel. In conclusion, the mobility formula of equation (1) explains well the surface properties of soft particles.

4. Surface structures of three types of non-uniformly charged latex particle covered with thermosensitive hydrogel layers

The electrophoretic mobility and size of three types of model latex particles were measured in [16]. The core particle was negatively charged and the outer layer, poly-NIPAM hydrogel, was either negatively charged or uncharged depending on the type of initiator used in the polymerization procedure, as schematically shown in figure 3. That is, sample A is the...
Figure 3. Schematic of latex particle preparation. Sample A is obtained first, using KPS as the initiator. It is then converted to Samples B and C by covering it with poly-NIPAM hydrogel layers, using KPS and VA-086 as the initiator, respectively. St, styrene.

‘core particle’ and is negatively charged. Samples B and C are the ‘core-shell particles’ prepared by covering sample A with a poly-NIPAM hydrogel layer. The surface layer of sample B has negative charges owing to the dissociated sulfate groups left at the polymer end, because potassium persulphate (KPS) was used as the initiator. On the other hand, the surface layer of sample C has no charges because we used 2,2′-azobis [2-methyl-N-(2-hydroxyethyl) propionamide] (VA-086) as the initiator. Sample B carries a negatively charged surface layer on the negatively charged core particle; however, sample C has an uncharged surface layer on the negatively charged core particle. These particles were monodisperse. The hydrodynamic particle size changed depending on the temperature of the suspending medium.

Figure 4 shows the observed relationship between the radius of the latex particles and the temperature of the suspending medium. A sudden change in the radius is observed in sample B around the phase transition temperature of poly-NIPAM, that is, the particle radius decreases by 18% from 570 nm at 20°C to 465 nm at 40°C. Most changes occur between 33 and 35°C where the radius decreases from 536 nm to 478 nm. In contrast, the radius is less temperature-sensitive in sample A, where it changes from 478 nm at 20°C to 428 nm at 40°C. As mentioned above, samples B and C contain sample A as the particle core and carry a layer of poly-NIPAM hydrogel on the surface. From a comparison of the temperature dependence of the radius between samples A and C, it is clear that the thickness of the poly-NIPAM hydrogel layer in sample C that covers the particle core (sample A) does not change appreciably with temperature, and that all changes occur in the particle core. However, the thickness of the poly-NIPAM hydrogel layer in sample B decreases from 93 nm at 20°C to 37 nm at 40°C. Therefore, both the core and poly-NIPAM hydrogel layer contribute to the temperature-dependent change in particle size in sample B.

To estimate the structural differences of the surface layers, we have measured the electrophoretic mobility at 25, 30, 33, 35 and 40°C, as shown in figures 5–7. The data,
Figure 6. Electrophoretic mobility of sample B as a function of ionic strength at pH 7.4 and the following temperatures: ◦, 25 ◦C; ♦, 30 ◦C; △, 33 ◦C; □, 35 ◦C; •, 40 ◦C. Solid curves are calculated with equation (1) using the following values: zN = 0.001 M and 1/λ = 2.8 nm at 25 ◦C; zN = −0.008 M and 1/λ = 2.4 nm at 33 ◦C; zN = −0.025 M and 1/λ = 1.5 nm at 35 ◦C, and zN = −0.056 M and 1/λ = 1.1 nm at 40 ◦C.

Figure 7. Electrophoretic mobility of sample C as a function of ionic strength at pH 7.4 and the following temperatures: ◦, 25 ◦C; ♦, 30 ◦C; △, 33 ◦C; □, 35 ◦C; •, 40 ◦C. Solid curves are calculated with equation (7) using the following values: zN = 0.01 M and 1/λ1 = 26 nm at 25 ◦C; zN = −0.065 M and 1/λ1 = 26 nm at 30 ◦C; zN = −0.072 M and 1/λ1 = 36 nm at 33 ◦C; zN = −0.078 M and 1/λ1 = 46 nm at 35 ◦C; zN = −0.09 M and 1/λ1 = 54 nm at 40 ◦C; 1/λ2 = 1.0 nm for all temperatures.

Figure 8. Charge densities zN of samples A (△) and B (◦) as functions of temperature.

Figure 9. Softness parameters 1/λ of samples A(△) and B(◦) as functions of temperature.

shown in figures 5 and 6, were analyzed with equation (1). Each theoretical curve with the associated pair of zN and 1/λ values fits well to the experimental data over a wide range of ionic strengths at each temperature. This means that sample A shows the electrophoretic behavior of a ‘soft particle’ described by equation (1) between 25 ◦C and 40 ◦C. Figures 8 and 9 show the variations in zN and 1/λ with temperature, respectively. The surface of sample A is negatively charged because KPS was used as the polymerization initiator. The negative charge density (zN) of sample A increases with temperature. This increase corresponds to thermosensitive shrinkage of sample A, as shown in figure 4. On the other hand, as seen in figure 9, the softness parameter 1/λ does not change with temperature in sample A, possibly because 90% of the polymer composing the particle is non-thermosensitive poly(styrene). Particles in sample B can be regarded as core-shell structures, in which the negatively charged core (sample A) is covered with a negatively charged poly-NIPAM.
show the values of with the same model as sample A. Also, in this case, each theoretical curve calculated via equation (1) fits well to the experimental data at 25, 33, 35 and 40 °C. At 30 °C, the electrophoretic mobility changed from slightly positive to negative as ionic strength increased, which might have been caused by the physisorption of sodium cations onto the surface layer. We observed a zero zN at 30 °C because the electrophoretic mobility should be negligible at any ionic strength; however, 1/λ was unknown. Figures 8 and 9 show the values of zN and 1/λ obtained from figure 6. A small change in zN is seen between 25 and 30 °C; however, the negative charge density increases from 0 to −0.056 M when temperature increases from 30 to 40 °C. Compared with sample A, sample B exhibited a clear effect of phase transition on zN. A strong decrease in 1/λ is seen between 33 and 35 °C, with a smaller decrease for 25–33 °C and 35–40 °C, indicating that sample B becomes harder as temperature increases. The sudden increase in charge density and hardness between 33 and 35 °C corresponds to the decrease in the thickness of the shell layer of sample B shown in figure 4.

When analyzing the data of sample C, we used a two-layer model in which the core particle with a charged soft layer is further covered with an uncharged soft layer, as shown in figure 10. This model is different from that used for samples A and B, which is essentially a one-layer model. The

Figures 7 and 11 show the calculated values of zN, 1/λ, Δ and 1/λ2 of sample C as functions of temperature. The associated equations are

\[
\mu = \frac{1}{\cosh(\lambda_1 d_1) + \frac{1}{\lambda_2} \sinh(\lambda_1 d_1)} \left[ \frac{\varepsilon_r \varepsilon_0}{\eta} \frac{\lambda_1}{\lambda_2^2} - k^2 \psi(-d_1) \right] \\
\times \left\{ \left[ k + \frac{\lambda_1^2}{\lambda_2^2} \right] e^{-\kappa d_1} \sinh(\lambda_1 d_1) + \lambda_1 \\
+ \frac{\lambda_1^2}{\lambda_2^2} (e^{-\kappa d_1} \cosh(\lambda_1 d_1) - 1) \right\} \\
+ \frac{zeN}{\eta \lambda_2^2} + \frac{\varepsilon_r \varepsilon_0}{\eta} \left[ \frac{\psi(-d_1)}{\lambda_1} - \frac{\psi(0)}{\lambda_2} \right].
\]  

Figure 11 shows the calculated values of zN, 1/λ, Δ and 1/λ2 of sample C as functions of temperature. The negative charge density (zN) in the inner layer (layer 2) increases with temperature, although 1/λ2 remains constant. Note that 1/λ1 increases with temperature revealing that the surface layer becomes softer. This behavior is opposite to that of sample B and might be caused by their different temperature-dependent structural changes. As revealed in figure 10, the shell thickness decreases with temperature in sample B, whereas it does not change in sample C. Thus, we conclude that in sample C, the uncharged polymer segments in

\[ \text{inner layer (sublayer 2)} \]

\[ \text{outer layer (sublayer 1)} \]

- 0.10
- 0.00
- 0.02
- 0.10
- 0.20

\[ 20 \ 25 \ 30 \ 35 \ 40 \ 45 \]

\[ 0 \ 10 \ 20 \ 30 \ 40 \ 50 \ 60 \]

\[ 1/\lambda_1 \text{ and } 1/\lambda_2 (\text{nm}) \]

\[ zN \text{ (M)} \]
the outer sublayer do not shrink in the direction perpendicular to the particle surface, but approach each other, making some surface patterns and voids, as shown in figure 12. This can decrease $1/\lambda_1$, decreasing the friction of liquid flow around the particles as temperature increases. This type of surface restructuring is possible when the polymer segments are uncharged and thus there is no electric repulsion. This phenomenon is not observed in sample B. This result agrees with the observation that the friction coefficient of water flow inside the poly-NIPAM gel layer decreases with temperature at a constant gel volume, as reported by Tokita and Tanaka [17]. They explain this observation as follows. Some portions of the gel swell and others shrink keeping the total gel volume constant, while water passes through the swollen open space avoiding the shrunk regions.

5. Electroosmosis on thermosensitive hydrogel surface

Electrophoretic mobility measurements are useful for studying the surface properties of colloidal particles dispersed in an electrolyte solution, but they cannot be applied to the study of charged plates. For such systems, electroosmotic velocity measurements or streaming potential measurements are most powerful. The former measurements cause less damage to the plate surface which is essential for a soft surface.

Electroosmosis measurements were applied to the estimation of the surface properties of a crosslinked poly-NIPAM hydrogel plate at different temperatures [18, 19]. The swelling ratio of the hydrogel at equilibrium in a phosphate buffer solution of pH 7.4 was measured as a function of temperature and ionic strength of the solution. The swelling ratio $W_s/W_p$ was defined as the weight ratio of adsorbed water ($W_s$) and the dry sample ($W_p$). The results are shown in figure 13. Phase transition is clearly seen at approximately $33^\circ$C; at lower temperatures the poly-NIPAM hydrogel is swollen and it is shrunk at higher temperatures. Generally, the equilibrium swelling ratio of a charged hydrogel is higher in a solution with lower ionic
strength, because of a stronger electrostatic repulsion among hydrogel-fixed charges. The swelling ratio of the poly-NIPAM hydrogel plate, however, exhibits a weak dependence on the ionic strength because of a small amount of fixed charges. The charge distribution in this poly-NIPAM hydrogel plate is expected to be uniform, and thus the ionized groups of valence $z$ should have a uniform density of $N$ (or the charge density of $zN$). In the present case, the ionized groups are sulfonic groups and $z = -1$. The charge density $zN$ can be calculated as the number of the sulfonic acid groups (which was measured potentiometrically as $10^{-4}$ mol g$^{-1}$) divided by the hydrogel volume. The hydrogel volume was calculated from $W_s/W_p$ at an ionic strength of 0.02 (figure 13). As mentioned above, the swelling ratio of the poly-NIPAM hydrogel plate hardly depends on the ionic strength. The results are given in figure 14, which shows that $zN$ changes from $-0.008$ M at 25°C to $-0.03$ M at 40°C.

The electroosmotic velocity ($U_{eo}$) per unit electric field ($E$) on the hydrogel surface immersed in an electrolyte solution was measured at pH 7.4 as a function of the ionic strength and temperature of the solution, and the results are shown in figure 15. The quantity $\mu = -U_{eo}/E$ is equal to the electrophoretic mobility as a result of the inverse relation between electrophoresis and electroosmosis. It is negative at all temperatures and ionic strengths. Its absolute value decreases with ionic strength and it does not reach zero even in a solution of very high ionic strength. Also, $\mu$ becomes more negative as temperature increases. We have analyzed experimental results with a theory of electro-osmosis on a ‘soft’ surface. In the hydrogel plate, the fixed charges are distributed uniformly, electrolyte ions in the bulk solution phase can penetrate the hydrogel layer, and the electrolyte solution can flow parallel to the surface up to some depth from the surface. Therefore, the surface of this hydrogel is expected to behave as a ‘soft surface’. Solid curves in figure 15 show the $\mu$ calculated with equation (1) as a function of electrolyte concentration. They fit well to the experimental data over a wide range of ionic strengths at each temperature. This means that between 25 and 40°C, the poly-NIPAM hydrogel surface can be considered a ‘soft surface’ described by equation (1) and the charges are distributed uniformly in the hydrogel at each temperature. Also, this agreement allows estimation of the values of the unknown parameters $N$ and 1/$\lambda$ through curve fitting. The best fit (solid lines in figure 14) was obtained with $zN = -0.008$ M and 1/$\lambda = 3$ nm at 25°C; $zN = -0.011$ M and 1/$\lambda = 3$ nm at 30°C; $zN = -0.015$ M and 1/$\lambda = 3$ nm at 33°C; $zN = -0.028$ M and 1/$\lambda = 2.1$ nm at 35°C; and $zN = -0.032$ M and 1/$\lambda = 1.9$ nm at 40°C. In the calculation, for the surrounding media, we used the viscosity $\eta$ and the relative permittivity $\varepsilon_r$ of distilled water at each temperature.

The charge density ($zN$) and softness parameter (1/$\lambda$) of the hydrogel are plotted as functions of temperature in figure 16. As mentioned above, poly-NIPAM hydrogel is negatively charged. The negative charge density of the hydrogel layer ($zN$) increases with temperature and this
increase is largest between 33 and 35 °C. Also, the surface of the hydrogel becomes harder (1/\(\lambda\) decreases) as temperature rises. The softness parameter changes abruptly between 33 and 35 °C and is almost constant above 35 °C and below 33 °C. By comparing these results with the temperature dependence of \(W_s/W_p\) between 25 and 33 °C, we found that the shrinkage of the hydrogel increases the charge density, but it has little effect on the friction of the liquid flow on the hydrogel surface. At 25 °C there are about 100 water molecules around one monomer unit of poly-NIPAM, and this number decreases to 50 at 30 °C, as calculated from the molecular weights of water and N-isopropylacrylamide (\(M_w = 113.17\)). The shrinkage of the hydrogel when the temperature rises from 25 to 33 °C squeezed out water molecules, thereby shortening the distance between polymer chains, while there is still sufficient space for the electrolyte solution to flow on the surface, and 1/\(\lambda\) is almost changed. As temperature rises, the hydrogel loses more water. At 35 °C one poly-NIPAM unit is surrounded by only about 25 water molecules, and the hydrogel becomes completely opaque. This decrease in the number of water molecules between 33 and 35 °C and the decrease in the distance between polymer chains result in a sharp drop in the friction coefficient of the hydrogel surface so that 1/\(\lambda\) decreases from 3 to 2.1 nm. Between 35 and 40 °C, no appreciable change is seen in \(W_s/W_p, zN\) or 1/\(\lambda\).

A comparison of figures 14 and 16 reveals that the \(zN\) values determined from the electroosmotic measurements agree with those calculated from the volume change of the hydrogel and the charge amount determined by potentiometric titration. Also, the temperature dependences of the charge density and softness of poly-NIPAM hydrogel estimated from the electroosmotic data correlate with the electrophoretic measurement results for poly-NIPAM-coated polystyrene latex surfaces, that is, the deduced \(zN\) and 1/\(\lambda\) values are on the same order of magnitude. The deviations may be caused by the difference in sample preparation conditions. We conclude that electroosmosis measurements appropriately enable the evaluation of the physicochemical properties of soft surfaces such as a hydrogel plate, which may serve as a model of a plate-like or membrane-like cell assembly.

6. Changes in electrophoretic mobility of HL-60RG cells induced by apoptosis

In this section and sections 7 and 8, we present the results obtained for various biological cells as described in [20–25, 28, 30, 32, 33]. The studies discussed in these references were stimulated by the availability of intact cells and their apoptotic or malignant modifications. Apoptosis is a form of cell death morphologically characterized by, for example, the condensation of the nuclear chromatin, the compaction of cytoplasmic organelles, the loss of surface microvilli and the blebbing of the plasma membrane [20–24]. Apoptosis is observed in normal physiological processes, such as an immunologically mediated negative selection of autoreactive T-cell clones and an unwanted cell elimination from tissues during embryogenesis; it is also induced by the exposure of cells to some toxins. Apoptosis can be selectively triggered by cells in response to some stimuli. It can result in the degradation of the nuclear DNA into oligonucleosome chains and in the fragmentation of the cells into neat ‘bite-size’ pieces for efficient disposal by neighboring cells or marauding macrophages. Because apoptotic cells interact specifically with phagocytic cells, the surface of apoptotic cells should be different from that of normal cells.

To study the apoptosis-related changes in the physicochemical properties of the cell surface, we have measured the electrophoretic mobility of intact and apoptotic human promyelocytic leukemia cell lines (HL-60RG cells). This quantity is expected to reflect certain properties of the surfaces of these cells and their change due to apoptosis [25]. Apoptosis was induced in HL-60 RG cells by actinomycin D treatment. DNA cleavage to soluble fragments, chromatin condensation and cell shrinkage were observed in order to confirm the apoptosis initiation. Figure 17 shows the results of electrophoretic mobility measurements in intact and actinomycin D-induced apoptotic HL-60RG cells redispersed in phosphate buffer solution, as a function of ionic strength at pH 7.4. No morphological changes were induced for intact and apoptotic cells in solutions with ionic strengths between 0.005 and 0.154. This implies that the presence of ions and sucrose, which is added to adjust osmotic pressure, does not significantly affect the cell structure during the measurements. Both types of cell exhibit negative mobilities, implying that the surfaces of these cells carry a net negative charge. The mobility is more negative for apoptotic HL-60RG cells than for intact HL-60RG cells at ionic strengths between 0.005 and 0.154.
The values of \( zN \) and \( 1/\lambda \) were deduced by fitting data using equation (1) as \( zN = -0.0336 \text{ M} \) and \( 1/\lambda = 1.7055 \text{ nm} \) for intact HL-60RG cells and as \( zN = -0.0346 \text{ M} \) and \( 1/\lambda = 1.7635 \text{ nm} \) for apoptotic HL-60RG cells. The values of \( zN \) are similar for both types of cell, while \( 1/\lambda \) increases upon apoptosis. In other words, the mobility difference between the intact and apoptotic HL-60RG cells is attributed mainly to \( 1/\lambda \) rather than the difference in the surface charge density. A larger \( 1/\lambda \) translates into a lower friction exerted by the polymer segments at the cell surface on the liquid flow. This increase in \( 1/\lambda \) can be caused by the increase in the ‘softness’ of the polymer chains or by their decrease in density. The predicted increase in the softness of the surface of apoptotic cells should correlate with the observation that apoptosis enhances cell deformability prior to cell fragmentation.

7. Differences in surface properties of malignant lymphosarcoma cell line RAW117-P and its variant H10

To study the changes in the physicochemical properties of the cell surface responding to a malignant behavior, we have measured the electrophoretic mobility of the malignant lymphosarcoma cell line RAW-117P and its variant H10 exhibiting a strong metastatic effect in the liver. The mobility was measured as a function of the ionic strength of the dispersant media [26–28]. Different values of \( zN \) and \( 1/\lambda \) were obtained for the RAW-117P and RAW-117-H10 cell surfaces, as shown in figure 18. The mobility is higher for the parental cell line (RAW117-P) than for its variant (RAW117-H10) for all electrolyte concentrations used. The best fit (solid lines in figure 18) to the experimental data was obtained with \( zN = -0.04 \text{ M} \) and \( 1/\lambda = 1.5 \text{ nm} \) for RAW117-P cells and with \( zN = -0.03 \text{ M} \) and \( 1/\lambda = 1.7 \text{ nm} \) for RAW117-H10 cells. The amount of sialic acid groups was about 27% smaller in RAW117-H10 cells than in RAW117-P cells, indicating that about 27% sialic acid was removed by the modification of RAW117-P cells.

The observed mobility difference between RAW117-P and RAW117-H10 cells is attributed to the unequal friction exerted by the cell surface layers on the liquid flow around the cells and to the difference in fixed-charge density between their surface layers. The surface charge density is lower and the surface is softer for RAW117-H10 cells than for RAW117-P cells. These data reflect certain properties of the cell surface and their change due to the malignant behavior. The induced ‘softness’ of the RAW117-H10 cell surface may be advantageous for metastatic tumor cells, allowing them to survive or adapt in a capillary with higher blood pressure and to invade the endothelial cells of the target tissue.
8. Change in electrokinetic properties of rat basophilic leukemia cell surface induced by cell adhesion onto solid surface

The electrokinetic surface properties of rat basophilic leukemia cells (RBL) were determined by electrophoresis and electroosmosis measurements. These cells are a useful model system for studying how the biosynthesis and secretion of inflammatory mediators, including peptide leukotrienes, are regulated in vivo [29, 30]. In general, basophilic cells can release peptide leukotrienes only after they have infiltrated into the extravascular space, where they adhere to various solid substrates such as collagen and fibronectin matrices [31]. The change in the electrokinetic properties of RBL cells upon the cells’ adhesion to a solid substrate (slide glass) has been studied. Free cells remained spherical, whereas the adhering cells spread over the glass surface to form a single monolayer. Furthermore, RBL cells can secrete a potent mediator, i.e. peptide leukotriene, when adhering to a solid substrate, including glass [32].

The electrophoretic mobility of the free RBL cells is plotted against the ionic strength of the dispersing medium in figure 19 [33]. They are negative at all ionic strengths in the pH 7.4 solution, implying that the surfaces of this type of cell have a net negative charge. The electrophoretic mobility changes from $-1.85$ to $-1.05 \text{ mm}^2 \text{s}^{-1} \text{V}^{-1}$ in the solution with ionic strengths between 0.01 and 0.154. The electrophoretic mobility has a non-zero value even in the solution with an ionic strength of 0.154. Fitting the data with equation (1) yielded $zN = -0.031 \text{ M}$ and $1/\lambda = 1.5 \text{ nm}$.

Electroosmosis measurements were used to characterize the electrokinetic surface properties of the adhered RBL cells, which covered the glass surface in a membrane-like cell assembly. The electroosmotic velocity ($U_{eo}$) per unit electric field ($E$) on the cell surface was measured at pH 7.4 as a function of ionic strength to evaluate the changes in the charge density ($zN$) and softness ($1/\lambda$) of the cell surface. The results are plotted in figure 19 against the ionic strength of the bulk solution. The mobility $\mu$ agrees well with the expression for the electrophoretic mobility of soft particles; it was negative at all ionic strengths, and its absolute value
decreases with ionic strength. The surface charge density (zN) of RBL cells changes from $-0.016$ to $-0.025$ M, and their softness parameter ($1/\lambda$) increases from 2.0 to 2.3 nm upon adhesion to glass. The RBL cells pass through the extravascular space and can release peptido leukotrienes only after infiltrating the extravascular space, where they adhere to various solid substrates such as collagen and fibronectin matrices. Thus, these changes in cell function and shape start when the RBL cells approach the endothelial cell surfaces.

9. Effects of lung surfactants on rifampicin release rate from monodisperse rifampicin-loaded PLGA microspheres

Tuberculosis is a chronic infectious disease caused by the infection by Mycobacterium tuberculosis. It is the major cause of death due to a single microorganism and its incidence is increasing, particularly in association with the AIDS pandemic [34–36]. Rifampicin is the first-choice drug in the treatment of tuberculosis. The treatment involves a prolonged and systematic oral administration of large doses of combined antibiotics that results in numerous side effects. Thus, new administration routes that minimize the side effects and enhance therapeutic effects are required [37–39].

We have prepared inhalable and monodisperse poly(lactide-co-glycolide) (PLGA) microspheres targeting tubercle bacilli residing in alveolar macrophages [38–40]. The effects of pulmonary surfactant on the rate of rifampicin (RFP) release from RFP-loaded poly (lactide-co-glycolide) microspheres were studied. The RFP release from RFP/PLGA microspheres was accelerated by the adsorption of pulmonary surfactants on the particle surface. The RFP release rate was faster for pulmonary surfactant-adsorbed PLGA particles in pH 7.4 buffer solution than for particles in pH 4.0 buffer solution or saline solution. The slowest release rate was observed when RFP/PLGA microspheres were dispersed in saline solution, although the release accelerated upon the addition of a pulmonary surfactant. From these results, we suggest that when RFP/PLGA microspheres are administered by inhalation, the RFP release rates are low for the particles that are not taken up by alveolar macrophages and that remain in the alveoli. The rate and amount of released RFP are higher after RFP/PLGA microspheres are taken up by alveolar macrophages existing in phagosomes, but they decrease after RFP/PLGA microspheres move into phagosome–lysosomes by the fusion of phagosomes with lysosomes. The surface properties of RFP-loaded PLGA microspheres were reported in [41, 42].

The absolute values of the electrophoretic mobility of PLGA microspheres increased upon the adsorption of pulmonary surfactants on the surfaces of PLGA microspheres, as shown in figure 20. The analysis of experimental data with the soft-particle model indicated that microspheres became ‘softer’ and the surface charge density of microspheres increased upon their degradation, as shown in figure 21. On the other hand, the surface of PLGA microspheres hardened and the electric charge density increased upon the adsorption of pulmonary surfactant on PLGA microspheres. The degradation-related changes in the surface charge density were enhanced by the adsorption of the lung surfactant on PLGA microspheres. It is presumed that the changes in surface properties of PLGA microspheres affect the uptake of these microspheres by alveolar macrophages.

10. Conclusion

In this review we have analyzed electrophoretic data of soft particles, such as biological cells and their model particles, using an electrokinetic theory of soft particles. This new theory differs from the conventional electrokinetic theory for hard particles in that it takes into account the surface structure of particles and thus depends on two parameters, that is, the density zN of fixed charges located in the surface layer of soft particles and the electrophoretic softness parameter $1/\lambda$. We have shown that these parameters characterize the detailed surface structure of soft particles and that it is possible to obtain new information on the surface properties of soft particles via the presented analysis.

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