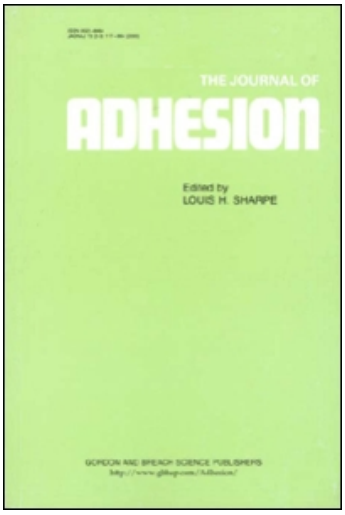

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A Particle Adhesion Perspective on Metastasis

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A Particle Adhesion Perspective on Metastasis

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One area of interest in bioadhesion that has not been emphasized within the adhesion science community relates to the disaggregation of cells which occurs when cancer metastases arise. Metastasis involves the distribution of cancerous tumor cells from a large localized tumor. The resulting spatial separation of the cancerous masses makes treatment more difficult. Making use of biochemical and cellular assays, detailed mechanisms for the cell detachment processes involving cadherin cell adhesion molecules have been proposed. This paper reviews proposed mechanisms for metastasis from a cellular adhesion perspective and the testing methodologies that have been utilized. Additional understanding might be gleaned from considering the loss of cell adhesion molecules and the overall disaggregation process from a particle adhesion perspective. Several pertinent theories are presented as well as a brief discussion of areas for future effort.

Keywords: Metastasis; Cadherin; DLVO theory; Cell adhesion

INTRODUCTION

Organized agglomerations of cells form the basis for mammalian tissue and organ architecture. Cellular regulatory mechanisms normally maintain a delicate balance of cell proliferation, quiescence, and death. This balance is disturbed in tumorigenesis. Tumor cells characteristically have exhibited increased growth and inhibited cell death,

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ultimately leading to an overall accumulation of tumor mass [1]. Malignant cells can detach from the growing mass and travel, *via* the bloodstream or lymphatic system, to distant sites where secondary tumors or metastatic lesions can be initiated (Fig. 1). Metastasis is a complex series of events involving a number of important factors; however, we will concentrate in this review on E-cadherin, one cell adhesion molecule thought to have a role in detachment from the growing tumor.

Detachment, whether active or simply the lack of cell adhesion, is a critical step in cancer progression [3]. Mammalian cells express (have present) a number of different adhesion molecules on their cell surface and the type and level are tightly regulated. The surface adherent molecules allow cells to bind specifically to particular cells and matrix components within the tissue, thereby promoting cell organization [4, 5] (see Fig. 2). Most tumors are derived from a specific type of cell known as an epithelial cell and the cadherin superfamily of adhesion molecules normally plays a role in epithelial cell connections [6]. Cadherin molecules span the cell membrane and their large extracellular domain can specifically interact with similar molecules on adjacent cells (Fig. 3). This is known as homotypic binding. E-cadherin, a member of the cadherin family involved in tight junction formation between neighboring epithelial cells, interacts with cell cytoskeletal components *via* molecules known as the catenins. This linkage

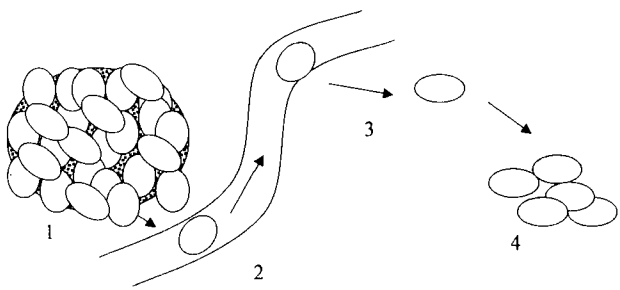

FIGURE 1 Metastasis is initiated when a cancer cell (1) detaches from the tumor mass, (2) enters the circulatory systems, (3) is transported to a distal site and invades the surrounding tissue, and (4) proliferates. Malignant tumors have this ability to leave the local environment and form metastatic lesions. It should be noted that a growing tumors mass establishes its own capillary network and that entry into the circulatory system need not occur outside the tumor itself.

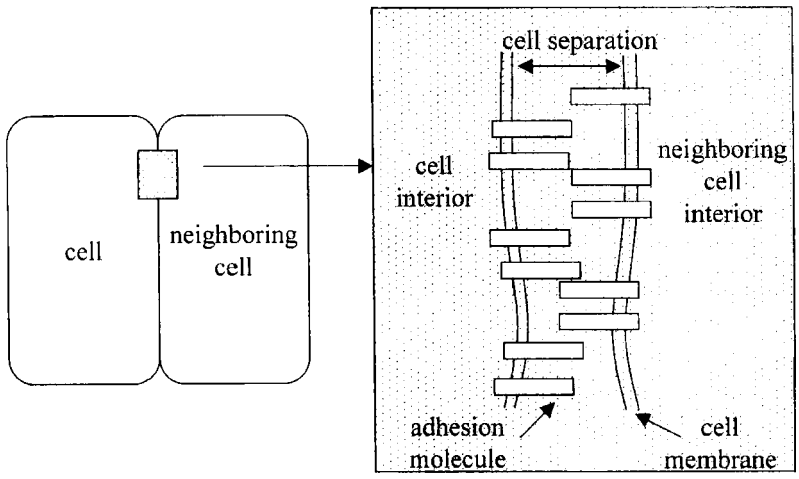

FIGURE 2 Changes in the synthesis or degradation rate of cell surface adhesion molecules can impact cell agglomeration. High levels of adhesion molecules increase the probability of cell binding and overall strength of attachment between neighboring cells. Reduced levels can result in poor adhesion due to both a reduction in overall bond strength and an increased probability of cell detachment.

to the cytoskeleton can provide rigidity and cell–cell attachment strength. Interruption of this linkage *via* changes in catenin interactions can lead to reduced cell adhesion [7, 8] and reduction in α-catenin may assist in tumor cell metastasis [9, 10]. In addition, altered expression of E-cadherin has been found in both primary and metastatic tumors from a number of different epithelial-derived cancers including pancreatic [11], prostate [12], gastric [13], and breast cancer [14, 15]. Further, E-cadherin expression and activity has been linked to growth factor stimulation, an additional potential link to tumorigenesis [16–18]. There is evidence that additional E-cadherin expression *via* cell transfection can reverse the invasiveness of malignant cells [19] although it has been suggested that the loss of E-cadherin, must be coupled with the expression of other cadherins in order to promote the proper environmental interactions needed by the invading cell [20]. There clearly exists a connection between overall metastatic activity and variations in the expression of specific adhesion and detachment factors with E-cadherin being an important example molecule.

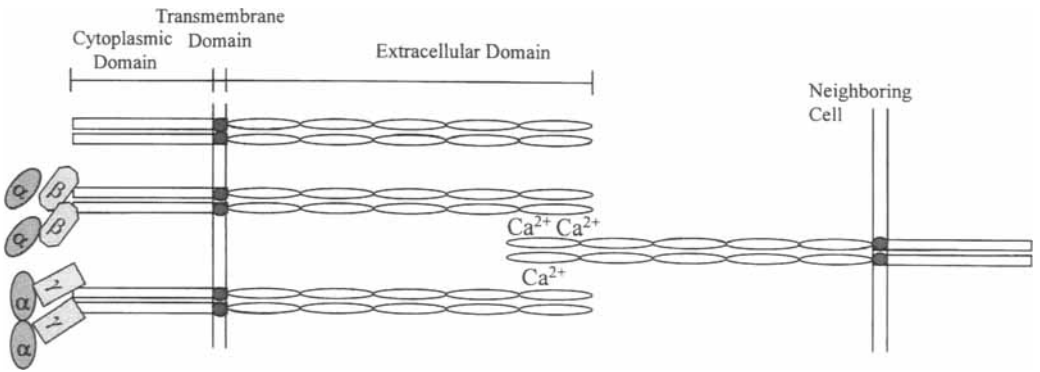

FIGURE 3 E-cadherin is a transmembrane molecule which forms homodimers in the presence of calcium. The extracellular domain contains 5 cadherin repeat units which can interact, in the presence of calcium, with E-cadherin molecules on neighboring cells. Homotypic (both E-cadherins) rather than heterotypic (E-cadherin binding with a different type of cadherin) adhesion is typical. The cytoplasmic tail of E-cadherin can interact directly with β-catenin (β) or γ-catenin (γ) which can then interact with α-catenin (α). Linkage to the actin cytoskeleton occurs through the catenin molecules and is required for cadherin-mediated cell adhesion.

Biological Adhesion Assays

Assessment of cell adhesion and detachment within the biological community is somewhat different from what one typically sees with a particle adhesion approach. Biological measurements of metastatic potential have focused on overall cell assays rather than specific bond measurements. For example, microscopy has been used to observe cell aggregation on surfaces [21, 22] and cell invasiveness into collagen gel [19]. Reaggregation assays have been frequently used in which agglomeration of dissociated cells is assayed using a Coulter Counter [21, 23, 24] (Fig. 4). Transfection of cells to express mutated E-cadherin [25] or α-catenin [7] or excess copies of E-cadherin [26] is another way in which the importance of these molecules with regard to aggregation have been studied. Alternatively, the resistance to cell removal by rinsing [7, 22, 27, 28] or centrifugation [8] is another way to assess cell adherence. These assays focus on the force required to eliminate cell association although, perhaps, a more direct analysis of binding could be made using a buoyancy-based assay which uses floatation to remove non-adherent cells [29]. In all cases, an overall rather than the individual interaction is being measured.

While some quantification can be associated with these biological assays, there is a limited ability to extract the true individual bond strength for cadherin interactions. Micromechanical methods to study individual cell–cell contact and deformation have been used extensively with blood cells to determine overall cell-adhesion energies [30–33]. Measurements of specific cell detachment based on surface interactions have been carried out using flow chambers to quantify the shear force required to cause cell detachment [34, 35]. Studies done under flow suggest that the heterogeneous nature of the cell glycocalyx, represented by antibody-coated beads, can affect detachment [36] and could play a role in cell detachment from a growing tumor. Recently, flow studies were performed to analyze the homotypic interaction between cadherin-coated surfaces and cadherin-coated spheres and, for example, an increase in binding frequency with cadherin surface density was observed [37]. Alternatively, optical tweezers are being investigated as tools for trapping both cells [38, 39] and individual molecules [40, 41] in order to evaluate the strength and specificity of the interaction. A recent paper by Sako *et al.*,

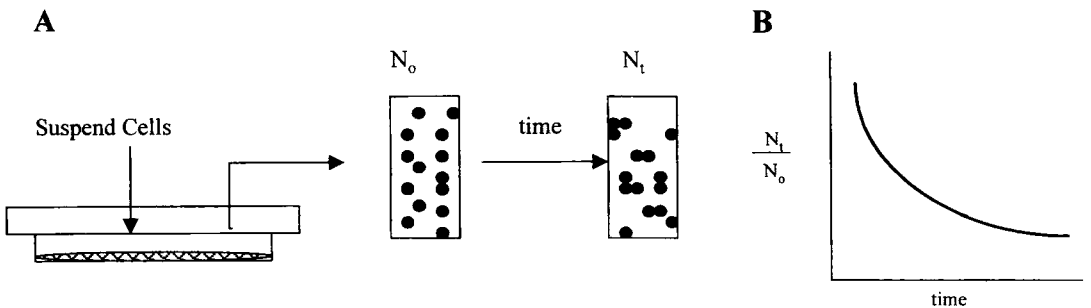

FIGURE 4 Reaggregation assay – (A) Cells are removed from the culture dish, generally *via* enzymatic dissociation, and resuspended in buffer. A sample is counted using a Coulter Counter to determine an initial number of cells (N_0). The solution is then incubated at 37°C on a shaker table to promote cell suspension and mixing. At time t, glutaraldehyde is added to fix the cells and a sample is counted in the Coulter Counter to determine the number of particles (N_t). (B) The amount of aggregation is determined from the reduction in particle number at time t compared to the original sample [24].

used laser trapping combined with particle tracking to evaluate diffusion of E-cadherin in the cell membrane and the role of cytoskeletal binding in retarding movement [42]. Finally, both atomic force microscopy (AFM) and the Surface Force Apparatus have been used to investigate molecular interactions and receptor-ligand binding and hold promise for quantifying adhesion binding exhibited by E-cadherin [43–45]. A challenge in evaluating the effect of specific cell adhesion molecules as they regulate cell adhesion is the interplay of other binding reactions, both extracellular and membrane-associated. The difficulty is in evaluating the relative contributions of each and how effective interventional strategies might be designed based on the pivotal associations.

CELL–CELL ASSOCIATION: A COLLOIDAL PERSPECTIVE

The agglomeration characteristics of cancer cells and the process of metastatic redistribution are of both clinical and fundamental interest and importance. Clinically, preventing deagglomeration of primary tumors could significantly ease surgical treatment and patient aftercare. Fundamentally, one would like to understand how attachment and detachment are regulated as these processes are important for tissue differentiation as well as metastasis. From an adhesion science perspective, the primary question is whether surface chemistry dominates binding across cell surfaces or if there is a structural lock-and-key effect controlled by specific cell adhesion molecules. This is a simplified picture, but one helpful for establishing a basic foundation.

Greater understanding can arise by linking experimental evidence with available theories. Three main approaches/theories exist to address attachment/detachment from a fundamental colloidal perspective. The first approach is to evaluate cell–cell interactions as colloidal agglomerations using some variation of the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory. The second approach evaluates particle interactions on surfaces making use of the Johnson–Kendall–Roberts (JKR) theory. The final approach is based on surface energetics.

DLVO Theory

DLVO theory relating colloidal stability of particles [46,47] has withstood the test of time as a generalized model to describe many problems in particle dynamics [48]. The theory has, as its basis, that particles in a medium, much like those dissolved in solution, change the chemical potential of the system. Surfactants, ions, pH, temperature and electrostatic interactions all can impact the relative isolation characteristics of each particle. As particle concentration increases, the number of cell–cell interactions that can be sensed by colligative properties like osmotic pressure increases [48,49]. Further, direct measurement of particle aggregation can be made using various scattering techniques such as X-rays [50], light [51,52], or neutrons [53]. The most important parameter that can be extracted from these types of measurements is the pair-correlation function, which gives the probability of finding the center of an interacting particle at a distance r from the center of a reference particle. This parameter is central to DLVO theory. The simplest DLVO structural analysis is based on spherical scattering sources (cell or particle) [54]; however, alternative scattering profiles have been determined for anisotropic particles [55,56], which may be more appropriate for certain cell types. The ability to test the theory fully for idealized particles or cells has improved, given that polymerization processes yielding large numbers of relatively spherical by-shaped particles are readily available [49]. Again, however, some heterogeneity both *in vivo* and *in vitro* with isolated mammalian cells is to be expected and will add uncertainty to theoretical predictions.

DLVO theory for particle interaction has been broken down along two main efforts primarily related to the relative stiffness of the interacting particles [48]. The classic hard-sphere model shows relatively small interactions in potential energy until contact between the particles occurs. Continued compression following contact leads to a rapid rise in the repulsive force pushing these particles apart. The deformation characteristics and membrane strength of mammalian cells confound use of this type of model, suggesting that soft sphere models would be more applicable to address cancer cell interactions. These models demonstrate a balance between the repulsive (steric and electrostatic) forces favoring separation and the attractive dispersion

forces favoring agglomeration. The potential barrier for agglomeration is the sum of these different potentials (Fig. 5).

With the soft-sphere model, the attractive dispersion force, $PE_{attractive}$, is given as:

$$PE_{attractive} = -\frac{A}{6}\left\{\frac{2a^2}{H(4a+H)} + \frac{2a^2}{(2a+H)^2} + \ln\left[\frac{H(4a+H)}{(2a+H)^2}\right]\right\}$$

where A is the Hamaker constant, typically in the range of 1×10^{-20} Joules, a is the particle radius and H is the distance between particles [57].

The corresponding repulsive force, $PE_{repulsive}$, comes from Overbeek [58, 59] and makes use of a moderate electrostatic potential. It is expressed as:

$$PE_{repulsive} = 2\pi a\varepsilon\varepsilon_0\left(\frac{4kT}{ze}\gamma\right)^2 e^{-\kappa H}$$

where κ is the Debye Parameter, H remains the interparticle distance, γ is a lumped parameter term, ε is the permittivity, ε_0 is the permittivity in vacuum, a is the particle radius, z is the ion valence, k is Boltzmann's constant, and e is the charge of an electron. Soft-sphere

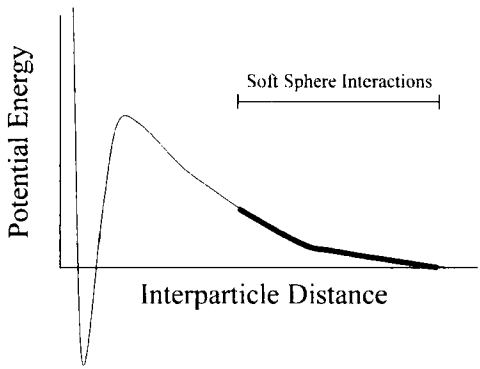

FIGURE 5 Potential energy diagram for soft-sphere interactions from a colloidal perspective. There are electrostatic interactions leading to repulsive forces between particles at relatively large interparticle spacings. There are also attractive interactions that favor interparticle wetting as the spacing decreases. Soft interactions can lead to cell–cell deformation which can increase repulsive forces if not capable of overcoming the potential energy barrier.

interaction models have been applied to latex particles and other colloidal dispersions as a function of pH and ionic strength of the suspension and appear to be appropriate for describing cellular interactions [60, 61]. Gingell and Fornes [62, 63] provided the first evidence of the validity of using DLVO in cell–cell interactions. Recent work by Molina-Bolivar *et al.*, has shown that the DLVO theory can be applied in a direct way to protein-mediated particle adhesion by interpreting the structure factors and clustering phenomena associated with protein-coated polymeric latex particles [57]. Evidence of the theory's validity is available; however, application to viable cells may prove difficult given the complexities of the normal cell glycocalyx interaction.

The independent variables associated with the theory relate primarily to the surface energy of the interaction and the charge associated with the colloidal interaction. These independent variables will define the interparticle spacing. To link theory with experiment, the goal would be to establish how changing the charge and surface energy of the cell mass leads to changes in the interparticle spacing between cells. In the event that stronger interactions beyond charge attraction are holding the cell mass together, varying charge and surface energy between cells in the mass will likely not lead to any observable change in the interparticle spacing. This would indicate that DLVO theory does not capture the essential phenomena and validates the importance of specific binding between adhesion molecules. Should interparticle spacing change appreciably with charge, it would indicate a reduced importance for molecules such as E-cadherin in the dissociation process, although this must be tempered with the knowledge that charge attractions may be involved in E-cadherin binding as well.

JKR Model

One could alternatively envision the detachment of a cell from a tumor mass as the loss of adhesion of a cell to a "tumor surface". There are a multitude of papers investigating particle-surface as well as cell-surface interactions [64–72]. Under this model, the interaction and corresponding adhesion of a particle to a surface is regulated by a series of contributions including the surface energetics of the

contact and the mechanical stiffness of the contacting media. JKR theory of particle contact on a solid surface has been applied to systems having larger surface energies and lower elastic moduli. Focus has been on determining for what regimes the theory is valid based on experimental studies of particles interacting on surfaces.

The theory is based on establishment of an equilibrium contact radius for the cell with the surface resulting from contributions relating to elastically-stored energy, the energy associated with surface forces of interaction, and the mechanical energy applied from an externally-applied load (Fig. 6). From a fundamental perspective, Quesnel *et al.* indicated that this theory is based on mathematical interpretation of particle adhesion, measured on a macroscopic level, with the physics of the interaction occurring on a microscopic or molecular level [73]. This is certainly the case for cellular adhesion, where cells may be idealized as spherical particles on the macroscopic level and textured surfaces with specific receptor proteins spanning the cell membrane on the molecular level.

JKR analysis predicts a relationship for this contact radius as [74, 75]:

$$a^3 = \frac{R}{K}[P + 3w_a\pi RP + (6w_a\pi RP + (3w_a\pi R)^2)]^{0.5}$$

where a is the contact radius formed from the particle wetting the surface, R is the particle radius, P is the applied load, w_a is the work

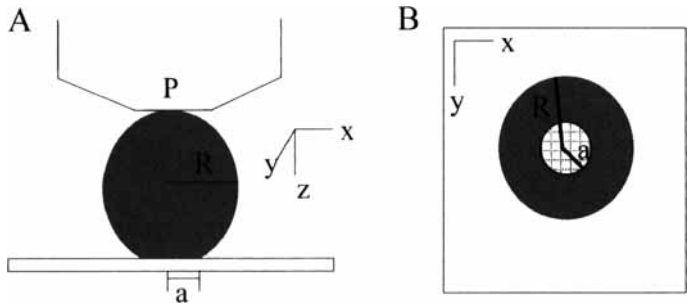

FIGURE 6 Particle adhesion configuration that considers the pressure-induced wetting of a hard sphere of radius R onto a soft substrate of known surface energy. Pressure-induced contact creates a circle of contact with radius, a, under applied load, P. The three-dimensional configuration of the apparatus is shown in part A and a two-dimensional projection looking up from the bottom of the apparatus is shown in part B.

of adhesion (a surface energy term), and K is an effective stiffness corresponding to the stiffness and Poisson's ratio of the constituents making up the particle/substrate interaction.

The work of adhesion or the energy and distance required to separate the particle from the surface can reasonably be represented by a Lennard-Jones Potential, where the exponents of the attractive and repulsive terms of the interaction are variables much like the energetics of molecular bonding [73]. This potential has been shown to represent polymeric interactions well in terms of their elastic properties and surface energy but has yet to be shown valid for a much more deformable body like a mammalian cell. For that matter, it is not clear whether all cell types would have similar surface energetics. These are gaps in knowledge which need to be explored.

One way to test the application of JKR theory to cell adhesion molecules and metastasis would be to spin-coat or deposit cell adhesion molecules onto a surface and then perform cell deformation experiments with the protein-coated surface. By controlling the force one applies, a cell or small tumor cluster could be brought into contact with the protein-coated surface and the contact radius measured as a function of the application pressure.These experiments could be done as a function of temperature or as a function of electrolyte concentration in the solution where both temperature and concentration should affect the "work of adhesion" term between cell and surface. Difficulties in performing these types of experiments are linked to the inelastic deformation of the cell during pressure application which may lead to altered force-contact curves at different locations of the cell. Further, at high pressures, cell rupture could occur. There may be other unexpected consequences for live cells due to the dynamic nature of the glycocalyx to pressure application, as well as other difficulties which may be due to variations in the orientation of the protein on the surface which may or may not be physiologically relevant. While the experiments are conceptually simple, data generated will need to be analyzed carefully to avoid inappropriate conclusions.

Surface Energetics of Cellular Interactions

Another interpretation for disaggregation occurring with metastasis could be made through the use of a surface energetics argument.

The surface energy of the agglomerated cell mass could be sufficient to allow for the detachment of smaller sections (metastases) which distribute and proliferate on their own. The application of surface energetics is well known in the biomaterials literature relating to eucaryotic cell adhesion to surfaces [76, 77]. Under this theory, cells interacting with a surface will spread if there is a thermodynamic driving force to increase cell-surface interactions. The process of cell wetting on a surface can be interpreted through contact angle measurements and has been elegantly described by the Young–Dupre equation [77–79]. Essentially, the contact angle is inversely related to the wettability of a particular surface and, as the cell spreads more onto a surface, an equilibrium develops. The amount of wetting has been related to the independent contributions of the surface energies of the cell and surface with the environment and the corresponding surface energy between cell and surface [77–79]. Spreading on the surface is a separate event following cell wetting and occurs over a much longer time scale. The difficulties in applying a surface energetics approach to metastasis are the inability to generate either measurements of the contact angle between cells *in vivo* and the lack of knowledge about what should represent the surface and its surface energy. Measurements could focus on whether tumor size impacts individual cell spreading and the ability of the cell to disengage from the mass. Another component that could be probed would be whether sectioned tumor masses exhibit the same level of cell–cell interaction or if adhesion is spatially dependent, particularly taking into account blood vessel location within the mass.

Application of Particle Aggregation Theory to Cadherins and Other Cell Adhesion Molecules in Cancer Metastasis

Biological aggregation is couched in very different language from colloidal adhesion theory; however, there is still tremendous overlap. Surface chemistry and morphology certainly play an important role in the fundamental phenomena in both sciences. However, the type and level of adhesion molecules present on the mammalian cell surface can change in response to environmental signals from neighboring cells as well as the extracellular matrix [80, 81], lending a complexity not generally seen in colloidal systems. In addition, the specific binding of cadherins and other adhesion molecules augments considerably

the attractive forces and decreases the probability of dissociation between the cells. These changes in the bond characteristics or number of bonds will likely impact the overall cell agglomeration.

There are a series of questions that need to be addressed to apply particle adhesion theory to cancer cell metastasis. The first is whether metastasis is a group/collective event or whether individual pairs of metastatic cells dissociate as well. It is conceivable that active metastasis may only occur when there is a large enough group of cells to trigger a reduction in the synthesis of cell adhesion molecules. Thus, experiments need to be conducted on cells in a wide range of densities to insure that the proper regime is investigated. Further, it is unlikely that cells will be homogeneous with regard to adhesion molecule levels and the issue of whether an isolated individual cell change is sufficient or if a global tumor change is needed must be examined. Therefore, while it may be simplistic, it is important to evaluate initially the effect of cadherin on cell aggregation and dissociation on uniform cells in isolation. *In vivo*, there are likely molecular and enzymatic cellular activities which can interact with cadherins and may increase the relative chance for metastasis or, alternatively, other adhesion molecules that could interfere with cadherin homotypic binding. Initial testing with coated polymeric beads and increasing the coating complexity incrementally would allow for the fundamental studies needed to analyze the complex cellular environment. Further, it would be advantageous to initiate studies in a fibrous or gelatin-like environment more representative of the tissue architecture. The lack of suitable isolation techniques and the cost for isolation of proteins such as E-cadherin can stymie these types of experimental efforts but does not diminish the need for these types of studies.

CONCLUSION

Cancer cell metastasis is a complex biochemical process, in which cell adhesive proteins affect the progression of the disease. Assays and flow cell measurements have been used to evaluate binding mechanisms and there are available theories that have been proposed that could be applied to cadherins and the process of metastasis.

The difficulty in applying theory to experiment in dealing with metastasis is an inherent problem in viewing behavior of an ensemble of particles relative to individual cell–cell interactions. "Colloidal" type experimental measurements can lend insight into the controlling phenomena of cell detachment and may facilitate the development of novel treatment protocols on a localized level.

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References

[1] Benitez-Bribiesca, L., In: *When Cells Die*, Lockshin, R. A., Zakeri, Z. and Tilly, J. L., Eds. (Wiley-Liss, New York, 1998), pp. 453–482.
[2] Hart, I. R., Goode, N. T. and Wilson, R. E., *Biochim. Biophys. Acta* **989**, 65–84 (1989).
[3] Shiozaki, H., Oka, H., Inoue, M., Tamura, S. and Monden, M., *Cancer* **77**, 1605–1613 (1996).
[4] Takeichi, M., *Science* **251**, 1451–1455 (1991).
[5] Petruzzelli, L., Takami, M. and Humes, H. D., *Am. J. Med.* **106**, 467–476 (1999).
[6] Christofori, G. and Semb, H., *Trends Biochem. Sci.* **24**, 73–76 (1999).
[7] Imamura, Y., Itoh, M., Maeno, Y., Tsukita, S. and Nagafuchi, A., *J. Cell Biol.* **144**, 1311–1322 (1999).
[8] Angres, B., Barth, A. and Nelson, W. J., *J. Cell Biol.* **134**, 549–557 (1996).
[9] Kadowaki, T., Shiozaki, H., Inoue, M., Tamura, S., Oka, H., Doki, Y., Iihara, K., Matsui, S., Iwazawa, T., Nagafuchi, A., Tsukita, S. and Mori, T., *Cancer Res.* **54**, 291–296 (1994).
[10] Shimoyama, Y., Nagafuchi, A., Fujita, S., Gotoh, M., Takeichi, M., Tsukita, S. and Hirohashi, S., *Cancer Res.* **52**, 5770–5774 (1992).
[11] Karayiannakis, A. J., Syrigos, K. N., Chatzigianni, E., Papanikolaou, S., Alexiou, D., Kalahanis, N., Rosenberg, T. and Bastounis, E., *Anticancer Res.* **18**, 4177–4180 (1998).
[12] Umbas, R., Schalken, J. A., Aalders, T. W., Carter, B. S., Karthaus, H. F. M., Schaafsma, H. E., Debruyne, F. M. J. and Isaacs, W. B., *Cancer Res.* **52**, 5104–5109 (1992).
[13] Mayer, B., Johnson, J. P., Leitl, F., Jauch, K. W., Heiss, M. M., Schildberg, F. W., Birchmeier, W. and Funke, I., *Cancer Res.* **53**, 1690–1695 (1993).
[14] Siitonen, S. M., Kononen, J. T., Helin, H. J., Rantala, I. S., Holli, K. A. and Isola, J. J., *Am. J. Clin. Pathol.* **105**, 394–402 (1996).
[15] Lipponen, P., Saarelainen, E., Ji, H., Aaltomaa, S. and Syrjanen, K., *J. Pathol.* **174**, 101–109 (1994).
[16] Al Moustafa, A. E., Yansouni, C., Alaoui-Jamali, M. A. and O'Connor-McCourt, M., *Clin. Cancer Res.* **5**, 681–686 (1999).

[17] Guvakova, M. A. and Surmacz, E., *Exp. Cell Res.* **231**, 149–162 (1997).
[18] Takahashi, K. and Suzuki, K., *Exp. Cell Res.* **226**, 214–222 (1996).
[19] Frixen, U. H., Behrens, J., Schs, M., Eberle, G., Voss, B., Warda, A., Lochner, D. and Birchmeier, W., *J. Cell Biol.* **113**, 173–185 (1991).
[20] Pishvaian, M. J., Feltes, C. M., Thompson, P., Bussemakers, M. J., Schalken, J. A. and Byers, S. W., *Cancer Res.* **59**, 947–952 (1999).
[21] Deman, J. J., Van Larebeke, N. A., Bruyneel, E. A., Bracke, M. E., Vermeulen, S. J., Vennekens, K. M. and Mareel, M. M., *In Vitro Cell. Dev. Biol.* **31**, 633–639 (1995).
[22] Komatsu, M., Carraway, C. A. C., Fregien, N. L. and Carraway, K. L., *J. Biol. Chem.* **272**, 33245–33254 (1997).
[23] Ozawa, M., Ringwald, M. and Kemler, R., *Proc. Natl. Acad. Sci. USA* **87**, 4246–4250 (1990).
[24] Takeichi, M., *J. Cell Biol.* **75**, 464–474 (1977).
[25] Nagafuchi, A. and Takeichi, M., *EMBO* **7**, 3679–3684 (1988).
[26] Nagafuchi, A., Shirayoshi, Y., Okazaki, K., Yasuda and Takeichi, M., *Nature* **329**, 341–343 (1987).
[27] Bauer, J. S., Schreiner, C. L., Giancotti, F. G., Ruoslahti, E. and Juliano, R. L., *J. Cell Biol.* **116**, 477–487 (1992).
[28] St. John, J. J., Schroen, D. J. and Cheung, H. T., *J. Immunol. Methods* **170**, 159–166 (1994).
[29] Goodwin, A. E. and Pauli, B. U., *J. Immunol. Methods* **187**, 213–219 (1995).
[30] Berk, D. and Evans, E., *Biophys. J.* **59**, 861–872 (1991).
[31] Evans, E., In: *Physical Basis of Cell–Cell Adhesion*, Bongrand, P., Ed. (CRC Press, Inc., Boca Raton, 1988), pp. 174–189.
[32] Tozeren, A., Sung, K. L., Sung, L. A., Dustin, M. L., Chan, P. Y., Springer, T. A. and Chien, S., *J. Cell Biol.* **116**, 997–1000 (1992).
[33] Shao, J. Y. and Hochmuth, R. M., *Biophys. J.* **71**, 2892–2901 (1996).
[34] Kuo, S. C. and Lauffenburger, D. A., *Biophys. J.* **65**, 2191–2200 (1993).
[35] Cozens-Roberts, C., Quinn, J. A. and Lauffenburger, D. A., *Biophys. J.* **58**, 857–872 (1990).
[36] Saterbak, A., Kuo, S. C. and Lauffenburger, D. A., *Biophys. J.* **65**, 243–252 (1993).
[37] Pierres, A., Benoleil, A. and Bongrand, P., *J. Phys. III* **6**, 807–824 (1996).
[38] Mammen, M., Helmerson, K., Kishore, R., Choi, S.-K., Phillips, W. D. and Whitesides, G. M., *Chem. Biol.* **3**, 757–763 (1996).
[39] Bronkhorst, P. J., Grimbergen, J., Brakenhoff, G. J., Heethaar, R. M. and Sixma, J. J., *Br. J. Haematol.* **96**, 256–258 (1997).
[40] Mehta, A. D., Rief, M., Spudich, A. D., Smith, D. A. and Simmons, R. M., *Science* **283**, 1689–1695 (1999).
[41] Finer, J. T., Mehta, A. D. and Spudih, J. A., *Biophys. J.* **68**, 291S–296S (1995).
[42] Sako, Y., Nagafuchi, A., Tsukita, S., Takeichi, M. and Kusumi, A., *J. Cell Biol.* **9**, 1227–1240.
[43] Hinterdorfer, P., Baumgartner, W., Gruber, H. J., Schilcher, K. and Schindler, H., *Proc. Natl. Acad. Sci. USA* **93**, 3477–3481 (1996).
[44] Wong, J. Y., Kuhl, T. L., Israelachvili, J. N., Mullah, N. and Zalipsky, S., *Science* **275**, 820–822 (1997).
[45] Willemsen, O. H., Snel, M. M., Kuipers, L., Figdor, C. G., Greve, J. and DeGrooth, B. G., *Biophys. J.* **76**, 716–724 (1999).
[46] Derjaguin, B. and Landau, L., *Acta Physicochim URSS* **14**, 633–662 (1941).
[47] Verwey, E. J. W. and Overbeek, J. T. G., *Theory of Stability of Lyophobic Colloids* (Elsevier, Amsterdam, 1948).
[48] Otterwill, R. H., *Faraday Discuss. Chem. Soc.* **90**, 1–15 (1990).
[49] Hunt, W. and Zukoswki, C., *J. Coll. Interf. Sci.* **210**, 332–342 (1999).
[50] Delfort, B., Daoudal, B. and Barre, L., *Tribol. T.* **42**, 296–302 (1999).
[51] Burns, J., Yan, Y., Jameson, G. and Biggs, S., *Langmuir* **13**, 6413–6420 (1997).

[52] Lehner, D., Worning, P., Fritz, G., Ogendal, L., Bauer, R. and Glatter, O., *J. Colloid Interf. Sci.* **213**, 445–456 (1999).
[53] Brunner-Popela, J., Mittelbach, R., Strey, R., Schubert, K., Kaler, E. and Glatter, O., *J. Chem. Phys.* **110**, 10623–10632 (1999).
[54] Debye, J. P., *J. Appl. Phys.* **15**, 338–342 (1944).
[55] Trinkhaus, J. P., *Cells into Organs, The Forces that Shape the Embryo* (Prentice Hall, Englewood Cliffs, NJ 1984).
[56] Cabannes, J. and Rochard, Y., *La Diffusion Moleculaire de la Lumiere* (Les Presses Universataires de France, Paris 1929).
[57] Molina-Bolivar, J., Galisto-Gonzalez, F. and Hidalgo-Alvarez, R., *J. Colloid Interf. Sci.* **208**, 445–454 (1998).
[58] Overbeek, J. T. G., *Adv. Colloid Interface Sci.* **16**, 17 (1982).
[59] Overbeek, J. T. G., *Pure Appl. Chem.* **52**, 1151 (1980).
[60] Kendall, K., Liang, W. and Stainton, C., *J. Adhesion* **67**, 97–109 (1998).
[61] Kendall, K., Liang, W. and Stainton, C., *Proc. Royal Soc. London A* **454** (1977), 2529–2533 (1998).
[62] Gingell, D. and Fornes, J. A., *Biophys. J.* **16**, 1131 (1976).
[63] Gingell, D. and Fornes, J. A., *Nature* **256**, 210 (1975).
[64] Bowers, V. M., Fisher, L. R. and Francis, G. W., *J. Biomed. Mat. Res.* **23**, 1453–1473 (1989).
[65] Bruil, A., Terlingen, J., Beugeling, T., van Aken, W. and Feijen, J., *Biomaterials* **13**, 915–923 (1992).
[66] Bruil, A., Brenneisen, L., Terlingen, J., Beugeling, T., van Aken, W. and Feijen, J., *J. Colloid Interf. Sci.* **165**, 72–81 (1994).
[67] Tirrell, M., Falsafi, A., Bates, F. and Pocius, A., In: *Proceedings of the Adhesion Society* **20**, 121–122, Hilton Head Island (1997).
[68] Scheuerman, T. R., Camper, A. K. and Hamilton, M. A., *J. Colloid Interf. Sci.* **208**, 23–33 (1998).
[69] Seyfert, S., Voigt, A. and Kabbeck-Kupijai, D., *Biomaterials* **16**, 201–207 (1995).
[70] Schakenraad, J. M. In: *Biomaterials Science – An Introduction to Materials in Medicine*, Ratner, B. D., Hoffman, A. S., Schoen, F. J. and Lemons, J. E., Eds. (Academic Press, San Diego, 1996), pp. 141–147.
[71] Tamada, Y. and Ikada, Y., *J. Colloid Interf. Sci.* **155**, 334–339 (1993).
[72] Brach, R., Li, X. and Dunn, P., *J. Adhesion* **69**, 181–200 (1999).
[73] Quesnel, D. J., Rimai, D. S. and DeMejo, L. P., *J. Adhesion* **67**, 235–257 (1998).
[74] Quesnel, D. J., Rimai, D. S., Gady, B. and DeMejo, L. P., In: *Proceedings of the Adhesion Society* **21**, 290–292, Savannah (1998).
[75] Johnson, K. L., Kendall, K. and Roberts, A. D., *Proc. R. Soc. London, Ser. A* **324**, 301–313 (1971).
[76] Ratner, B. D., In: *Biomaterials Science – An Introduction to Materials in Medicine*, Ratner, B. D., Hoffman, A. S., Schoen, F. J. and Lemons, J. E., Eds. (Academic Press, San Diego, 1996), pp. 21–35.
[77] Schakenraad, J. M., Busscher, H. J., Wildevuur, C. R. H. and Arends, J., *Cell Biophyics* **13**, 75–91 (1988).
[78] van Wachem, P. B., Beugeling, T., Feijen, J., Bantjes, A., Detmers, J. P. and van Aken, W. G., *Biomaterials* **6**, 403–408 (1985).
[79] Jansen, J. A., van der Waerden, J. P. C. M. and de Groot, K., *Biomaterials* **10**, 604–608 (1989).
[80] Dejana, E., *J. Clin. Invest.* **98**, 1949–1953 (1996).
[81] Bongrand, P., Capo, C., Mege, J.-L. and Benoliel, A.-M., In: *Physical Basis of Cell–Cell Adhesion*, Bongrand, P., Ed. (CRC Press, Boca Raton, 1988), pp. 61–90.