Indian Journal of Experimental Biology
Vol. 45, January 2007, pp. 9-17

RBC aggregation: Laboratory data and models

H J Meiselman[1]*, B Neu[2], M W Rampling[3] & O K Baskurt[4]

[1]Department of Physiology and Biophysics, Keck School of Medicine, 1333 San Pablo Street, MMR 626 Los Angeles, CA 90033, USA

[2] School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore

[3]Imperial College School of Medicine, London SW7 2AZ, UK

[4]Department of Physiology, Akdeniz University School of Medicine, Antalya, Turkey

Received 5 June 2006

The reversible aggregation of red blood cells (RBC) into linear and three-dimensional structures continues to be of basic science and clinical interest: RBC aggregation affects low shear blood viscosity and microvascular flow dynamics, and can be markedly enhanced in several clinical states. Until fairly recently, most research efforts were focused on relations between suspending medium composition (i.e., protein levels, polymer type and concentration) and aggregate formation. However, there is now an increasing amount of experimental evidence indicating that RBC cellular properties can markedly affect aggregation, with the term "RBC aggregability" coined to describe the cell's intrinsic tendency to aggregate. Variations of aggregability can be large, with some changes of aggregation substantially greater than those resulting from pathologic states. The present review provides a brief overview of this topic, and includes such areas as donor-to-donor variations, polymer-plasma correlations, effects of RBC age, effects of enzymatic treatment, and current developments related to the mechanisms involved in RBC aggregation.

Keywords: Aggregation, RBC

Erythrocytes in static human blood form loose aggregates with characteristic face-to-face morphology, similar to a stack of coins. Such aggregation is frequently referred to as rouleaux formation, and is caused by the presence in plasma of a variety of macromolecules, especially fibrinogen. Similar aggregation can be caused by suspending RBC in solutions of high molecular weight water-soluble polymers (e.g., dextran, poly(ethylene glycol), polyvinylpyrrolidone). In normal, non-pathologic blood, aggregates break up when subjected to relatively low shear rates (e.g., 20-40 s^{-1}), suggesting relatively weak attractive forces; greater cell-cell attractive forces can exist in pathologic blood or for RBC aggregated by large polymers. It is notable that RBC aggregation requires the presence of an "aggregant" in the suspending medium since aggregates do not form if cells are washed and re-suspended in protein- or polymer-free saline or buffer.

RBC aggregation is important because it is largely responsible for the profound shear thinning behavior of normal human blood (i.e., decreasing blood viscosity with increasing shear rate due to dispersion of aggregates). RBC aggregation also has substantial effects on *in vivo* hemodynamics and can adversely affect red blood cell distribution and flow dynamics in small tubes and the microcirculation[1-6]. Further, the level of aggregation can rise enormously in association with a wide variety of clinical conditions such as sepsis[7], diabetes[8], myocardial ischemia[9] and renal failure[10]. However, in spite of extensive literature dealing with RBC aggregation, there are still many areas where additional information is needed. In particular, information related to the mechanism or mechanisms that are responsible for aggregation is insufficient. Two mechanistic models currently exist (i.e., cross-bridging and depletion layer[11]). However, neither of these approaches has received universal acceptance, most likely because conclusive evidence has yet to appear. However, a relatively new area of investigation, the study of cell-specific factors that influence red cell aggregation, is exposing novel information that should aid in resolving this dilemma.

Work in the area of cellular factors affecting RBC aggregation has been prompted by the salient observation of Nordt[12] who showed that density-separated (i.e., age-separated) RBC exhibited

Correspondent author:
Phone: 323-442-1268
FAX : 323-442-2283
E-mail: meiselma@usc.edu

different degrees of aggregation when suspended in autologous plasma: older, more dense cells exhibited greater aggregation. This observation has been confirmed, and correlations between RBC aggregation in autologous plasma and in standard polymer solutions have been demonstrated[13,14]. These results indicate that there is a large variation in the aggregating potential of cells from different normal, healthy subjects: high responders to plasma also tend to be high responders to dextran, whereas low responders in one medium are low in the other. Hence, there are intrinsic cell-specific factors that control the intrinsic aggregability of red cells, with these factors differing greatly between individuals. Note that previously all RBC were tacitly assumed to respond equally to a given aggregant: differences between subjects for cells in plasma were assumed to be due only to differences in plasma protein levels, with donor differences for cells in the same polymer solution were usually ascribed to experimental error or measurement variability.

Donor-specific differences (Adult donors)

The first major report in this area was that of Sowemimo-Coker *et al.*[14], who, studied erythrocytes from different healthy human adult subjects, suspended in autologous plasma or, after washing the cells free of plasma proteins, in phosphate buffered saline containing 3% dextran 70 (MW = 73 kDa). Paired data (e.g., extent of aggregation at stasis in plasma and in dextran) for each subject were then plotted against one another, with typical results shown in Fig. 1.

Data such as those shown in Fig. 1 are deemed important for two reasons:

(1) They show that the difference in aggregation tendency between subjects is large, approximately two-fold in the dextran solution and five–fold in autologous plasma. Such ranges are far beyond the approximately 3-7% variation associated with experimental and instrumental uncertainty. While the range of values for cells in plasma reflects contributions from both plasmatic (e.g., fibrinogen levels) and cellular factors, the range for dextran data can *only* be explained by subject-to-subject variations in cell-specific factors; (2) They show that high responders to dextran tend to be high responders to autologous plasma and vice versa (i.e., a linear correlation between aggregation in the two media, $P<0.001$). This finding suggests that cell-specific factors may be of similar significance for any aggregating agent.

Experimental evidence supporting the above suggestion has been presented by Whittingstall *et al.*[15], who investigated RBC aggregation induced by phosphate buffered saline solutions of 360 kDa polyvinylpyrrolidone, 61.2 kDa poly-l-glutamic acid and 17 kDa sodium heparin as well as 70 kDa dextran and autologous plasma. For each of the four polymers the extent of aggregation produced by the aggregant was significantly correlated with that produced by the plasma ($r > 0.7$, $P < 0.01$). Their results thus indicate that in spite of differences in size and charge of the molecules (dextran and PVP are neutral while P-L-Glu and heparin are negative), aggregation induced by each aggregant exhibits a significant correlation with that induced by plasma; correlations were also found between aggregation by various polymer aggregants. Similar results have been reported by Rampling *et al.*[16] using various polymers and three separate approaches to the measurement of RBC aggregation.

Cellular factors for neonatal RBC

Several reports[11,16,17] have indicated that RBC from normal term neonates in autologous plasma exhibit significantly less aggregation than adult blood. Further, such aggregation depends on gestational age, in that blood from infants with 24 to 28 weeks of gestation does not show significant RBC aggregation[16]. RBC aggregation behavior of both preterm and term blood has been postulated to be due to increases in plasma protein levels and to the degree

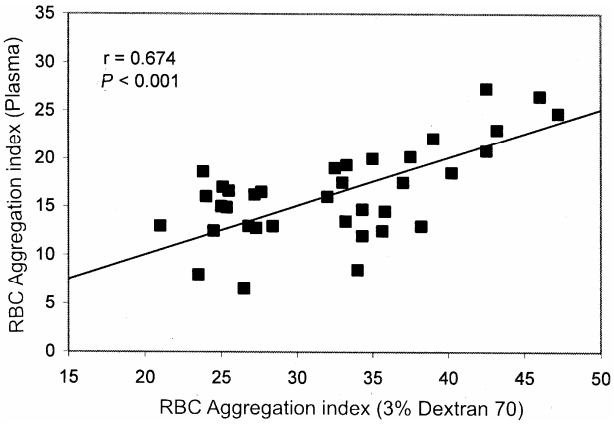

Fig. 1—Paired aggregation data for RBC from normal subjects suspended in autologous plasma or washed and re-suspended in 3% dextran 70. Straight line through data obtained by least squares linear regression ($P<0.001$) [modified from 47]

of sialination of fibrinogen with gestational age[18]: neonates generally have lower fibrinogen levels than adults and have hyper-sialinated fibrinogen.

Results dealing with the aggregation behavior of neonatal RBC in polymer solutions are more complicated than for adults since neonatal RBC aggregation appears to depend critically upon the specific polymer: (1) Using washed cells suspended in 500 kDa dextran, Linderkamp *et al.*[16] indicate no differences in rate or extent of aggregation from adult cells regardless of gestational age, and that neonatal RBC in adult plasma showed aggregation similar to that for adult cells in the plasma; (2) studies of term neonatal RBC in 70 kDa weight dextran indicate marked and highly significant differences from adult cells, with a 35% lower extent and a 60% lower strength of aggregation for the neonatal cells[19]. These authors[19] confirmed that 500 kDa dextran caused equal aggregation for neonatal and adult RBC, but only up to 1 g/dL, with neonatal cells having significantly lower aggregation at higher dextran 500 levels.

Cellular factors for various mammals

Johnn *et al.*[20] indicated large differences in the degree of RBC aggregation for RBC-plasma suspensions of different mammals; horse, leopard and rhinoceros exhibit very strong aggregation, no aggregation is observed for cattle, sheep and goat, with other mammals demonstrating intermediate behavior. Species-specific differences for cells in autologous plasma have also been reported[14,21,22]. Studies of aggregation in various polymer solutions have demonstrated differences between species: rat red blood cells exhibit low aggregation in concentrations of 70 kDa dextran that strongly aggregate human RBC, whereas bovine cells show negligible aggregation in 70, 500 or 2000 kDa dextran or in 360 kDa PVP[14]. Baskurt *et al.*[23] compared the blood of horse, human and rat since these species exhibit very high, medium and low aggregating tendencies in autologous plasma: a very clear pattern emerged in that horse had a high response in both plasma and dextran, the rat was low in both and human was intermediate. Similar patterns have been reported for horse, bovine and human cells re-suspended in 464 kDa dextran kDa[22] or for cells from these species re-suspended in fibrinogen, 70 kDa dextran, heparin or 360 kDa PVP[24]. Marine mammals also demonstrate cell-specific effects, in that blood from Weddell seals (*L. weddelli*) and bowhead whales exhibits intense RBC aggregation in autologous plasma and in solutions of various polymers[25].

Effects of cell age

Nordt[12] appears to have been the first to report the effects of RBC *in vivo* age on aggregation. He tested age-separated cells in autologous plasma and observed that the 10% densest cells had more than two-fold greater aggregation versus the 10% least dense cells, with cells of intermediate density exhibiting aggregation between these limits. Nash *et al.*[13] extended the observations of Nordt to studies of age-separated RBC re-suspended in solutions of 70 kDa dextran. These authors[13] also observed two-fold greater aggregation in plasma for older versus younger cells; similar results were obtained for cells washed and re-suspended in 70 kDa dextran (Fig. 2). The results shown in Fig. 2 are therefore consistent with those for un-fractionated cells shown in Fig. 1, in that enhanced aggregation in plasma predicts enhanced aggregation in 70 kDa dextran. Whittingstall, *et al.*[15] tested age-separated RBC in plasma, neutral polymers 70 kDa dextran and 360 kDa PVP, and in negatively charged polymers (i.e, 61.2 kDa poly-l-glutamic acid and sodium heparin), and observed greater aggregation for older cells in plasma and in all polymers. Thus, for both the plasma protein fibrinogen and for neutral and negatively charged polymers, denser, older RBC exhibit significantly greater extent and strength of aggregation compared to less dense, younger cells.

Given that neonatal RBC have much shorter *in vivo* lifespan (i.e., 60 days for neonates *vs* 120 days for adults), an obvious extension of the abovementioned

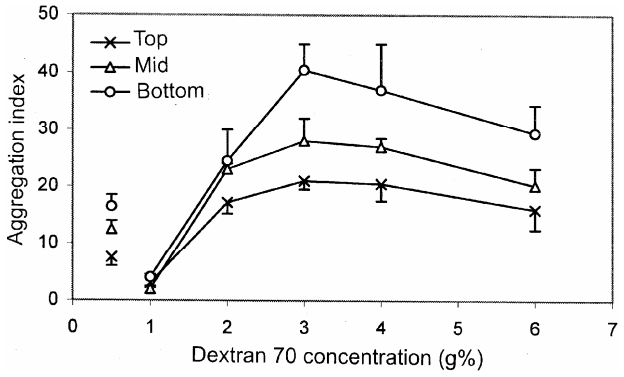

Fig. 2—RBC aggregation, as the Myrenne Aggregometer Aggregation Index, versus dextran T70 concentration for density-fractionated red cell suspensions. Data for cells suspended in *autologous plasma* are shown in the lower left and are arbitrarily positioned at the 0.5% location on the ordinate. (mean ± SD, n= 3)

studies employing adult cells is the study of neonatal RBC aggregation. Using a density-separation protocol identical to that employed for adult cells and 70 kDa dextran as the aggregant, it has been observed[11] that both neonatal and adult RBC have a older/younger aggregation ratio significantly greater than unity (Fig. 3). The ratio for neonatal RBC, however, is markedly less than for adult RBC, and no significant difference (P>0.5) was observed between younger neonatal and adult cells, whereas this difference was highly significant for older neonatal versus adult cells. While the results in Fig. 3 suggest that the length of time RBC are exposed to the *in vivo* circulatory environment may affect their aggregation tendency, other possibilities should be kept in mind since neonatal RBC are more prone to oxidant damage, have increased mechanical fragility, and some strongly age-dependent RBC enzymes decline more rapidly in neonatal red blood cells [26].

Effects of disease on clinical states

The body of literature dealing with RBC aggregation in various clinical states and diseases is voluminous, with most reports indicating enhanced aggregation for cells suspended in autologous plasma[27-29]. These enhanced levels of RBC aggregation, coupled with the abovementioned associations between plasma- and polymer-induced aggregation, raise the following question: is inherent RBC aggregability altered in clinical states and diseases characterized by abnormal RBC aggregation in plasma? Answers to the question include: (1) increased RBC aggregation induced by plasma and 70 kDa dextran in subjects with diabetes mellitus and Hansen's disease[11]; (2) in poorly controlled type 2 diabetes, RBC aggregation in plasma is significantly elevated, yet no differences from control were noted for cells re-suspended in a solution of 110 kDa dextran[10]; (3) in a study of adult type 2 diabetic subjects using diet, exercise and insulin to improve glycemic control, Chong-Marteinez, *et al.*[31] reported that RBC aggregation in *both* plasma and 70 kDa dextran was initially above control, yet significantly decreased in both media over a 14 week treatment period. Interestingly, levels of glycated hemoglobin (HbA1c) also decreased over this period, yet decreases of RBC aggregation in plasma or dextran (i.e., decreased red blood cell aggregability) did not correlate with the decreases of HbA1c [31].

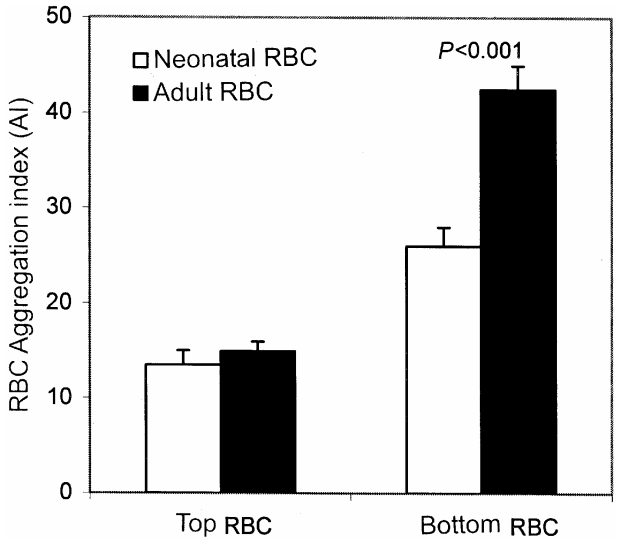

Fig. 3—Aggregation behavior of least-dense (i.e., TOP) and most-dense (i.e., BOT) RBC from full term neonates and from adults in 3% dextran 70.

Various rat-based experimental models have been employed to evaluate changes of cell-specific effects consequent to abnormal physiological states. These studies thus represent attempts to explore pathophysiology-aggregability relations using well-recognized protocols that approximate human diseases or clinical states. Hacioglu *et al.*[32] investigated RBC changes in rats made hypertensive by four different methods, then tested RBC aggregation in plasma and 500 kDa dextran. Their results indicate that the various methods for inducing hypertension produced differing degrees of RBC aggregation tendency and that, in general, manipulations altered RBC aggregation tendency whether the cells were suspended in plasma or 500 kDa dextran.

Kayar *et al.*[33] have examined the effects of ischemia and reperfusion of rat hind limb on red cell aggregation in samples obtained from the vein draining the ischemic limb. RBC aggregation was not affected by 10 min ischemia alone, but was significantly reduced following 15 min of reperfusion. This decrease was of equal magnitude for RBC in plasma or in 0.5% 500 kDa dextran, again implying cell-specific effects. The effects of experimental sepsis on RBC aggregation have also been investigated using a cecal ligation/puncture protocol in rats[7]; sham operated animals (i.e., laparotomized but no cecal ligation/puncture) and controls were also employed. After 18 hr of ligation/puncture, RBC aggregation in plasma was slightly increased for the sham animals and markedly increased for the septic ones, with the magnitude of the increases paralleling

increases of plasma fibrinogen levels. However, for RBC in 70 kDa dextran, RBC aggregation increased *only* for the septic animals[7]. Thus, the increases of aggregation seen in plasma for cells from sham and septic animals reflect both plasmatic and cellular influences, whereas the increase in the dextran medium is specific to cellular factors.

Mechanisms for RBC aggregation

As mentioned earlier, there are presently two co-existing yet mutually exclusive "models" for RBC aggregation, and it is of interest to consider these models vis-à-vis cellular properties affecting red blood cell aggregation.

Bridging model: Red cell aggregation via this mechanism occurs when the bridging forces due to the adsorption of macromolecules onto adjacent cell surfaces exceeds disaggregation forces due to electrostatic repulsion, membrane strain and mechanical shearing[34-37]. Chien has formalized this model via a force balance equation[35], and data for surface adsorption of dextrans and intercellular separation as a function of dextran molecular weight have been published[35]; Lominadze and Dean[38] have reported data indicating a specific binding mechanism between fibrinogen and rat RBC. Brooks and co-workers favor the bridging model for dextrans given their molecular weight threshold and adsorption isotherms, and because the free-energy reduction associated with adsorption is available to do the work required to aggregate the cells[34,39]. Conversely, Armstrong *et al.*[40] present experimental data using polymers co-valently attached to the RBC surface that argues strongly against macromolecular bridging as the mechanism for red blood cell aggregation induced by nonionic polymers.

Depletion model: Red cell aggregation via this mechanism occurs due to a lower protein or polymer concentration near the cell surface versus the suspending medium (i.e., relative depletion near cell surface). This preferential exclusion of macromolecules leads to an osmotic gradient and movement of fluid away from the intercellular gap, thereby promoting aggregation by decreasing cell-solvent affinity. The basic aspects of depletion-induced particle aggregation and its application to colloidal systems have been reviewed by de Gennes[41], and its applicability to red cell aggregation have been presented by van Oss *et al.*[42] and Evans *et al*[43].

The depletion model does not require an absolute lack of adsorption on the red cell in order to favor aggregation, but only that the adsorbed level be less than the bulk solution; a decrease or reversal of this gradient leads to decreased or abolished RBC aggregation[41,42].

The abovementioned two models are in conflict; the bridging model predicts increased aggregation consequent to increased protein or polymer concentration at the RBC surface, whereas the depletion model predicts the opposite. While direct measurement of adsorbed amounts of macromolecules per cell would aid in resolving this dilemma, an extensive review of literature values by Janzen and Brooks[39] has detailed likely technical artifacts (e.g., trapped fluid between RBC) and thus the extremely wide range of reported data for fibrinogen and dextran binding. However, an alternative approach has been developed by Baumler *et al.*[43,44]. These investigators studied RBC electrophoretic mobility (EPM) behavior in various dextran solutions, and have pointed out that because electro-osmotic flow obeys potentiality outside the diffuse double layer, the relevant viscosity is that within the double layer[44]. Supporting evidence for the depletion effect in electrophoresis has been reported by Arnold *et al.* [45], and Baumler *et al.*[43,44] have provided experimental approaches to the estimation of fluid viscosity and thus macromolecule concentrations near the cell and report a 10-fold reduction of dextran concentration near the RBC surface.

Recent publications by Neu and Meiselman[46] have materially advanced the understanding of the polymer depletion effect near the RBC surface. Studies directed toward validating the existence of the depletion layer employed measurements of unit-gravity cell sedimentation and of cell electrophoretic mobility (EPM) for RBC in various polymer solutions[46]. These studies were designed to test the hypothesis that regardless of polymer molecular weight, the effects of suspending medium viscosity on sedimentation could be predicted via the Stokes Equation, whereas EPM would become less sensitive to medium viscosity with increasing molecular weight (i.e., with increasing depletion layer thickness). As shown in Fig. 4, their experimental results support the hypothesis: the Stokes Equation is applicable regardless of polymer weight, whereas EPM follows the expected inverse relation[22] only for small polymers (i.e., 10 kDa dextran) and is essentially

independent of medium viscosity for large polymers (i.e., 500 kDa dextran). The latter point is of particular interest in that it demonstrates the existence of the depletion layer and its dependence on molecular size[46].

A theoretical model for depletion-mediated red cell aggregation has been developed[47] in which two opposing forces are considered: (1) an attractive force between cells due to an osmotic gradient (i.e., a chemical potential gradient) between the lower polymer concentration in the depletion layer and hence the intracellular gap, and the higher polymer concentration in the bulk phase; (2) an electrostatic repulsive force due to the negative charge on the RBC surface. The model considered that the concentration profile and hence the extent of the depletion layer was dependent on polymer size and concentration, glycocalyx thickness, and glycocalyx-polymer interactions (i.e., penetration of the polymer into the glycocalyx)[47]. Figure 5 summarizes major aspects of the model for various molecular weight dextran fractions. Note that the results shown in Fig. 5 are consistent with experimental measurements of RBC aggregation in dextran solutions[13,23,35], in that both a lower molecular weight threshold for aggregation and biphasic responses to increasing polymer concentration have been experimentally demonstrated. Note also that the agreement between theoretical predictions and experimental findings provides a basis for exploring the effects of altered glycocalyx properties on red cell aggregability.

As noted above, age-separated RBC exhibit differences in aggregability in either plasma or polymer solutions (i.e., older > younger), yet the differences are not related to altered cell volume, deformability or surface IgG levels. However, electrophoretic mobility studies of age-separated RBC have revealed an interesting pattern; no difference of EPM for cells in protein-free or polymer-free media, yet a small (≈5%) but significant greater mobility for older cells in plasma, serum or dextran solutions[11,14]. Baskurt *et al.*[48] have presented similar aggregation-EPM trends for RBC from healthy and septic rats: (1) no EPM differences when suspended in polymer-free buffer; (2) a small but significantly lower EPM for septic cells in a dextran 500 solution; and (3) markedly greater aggregation for septic cells in plasma or dextran 500.

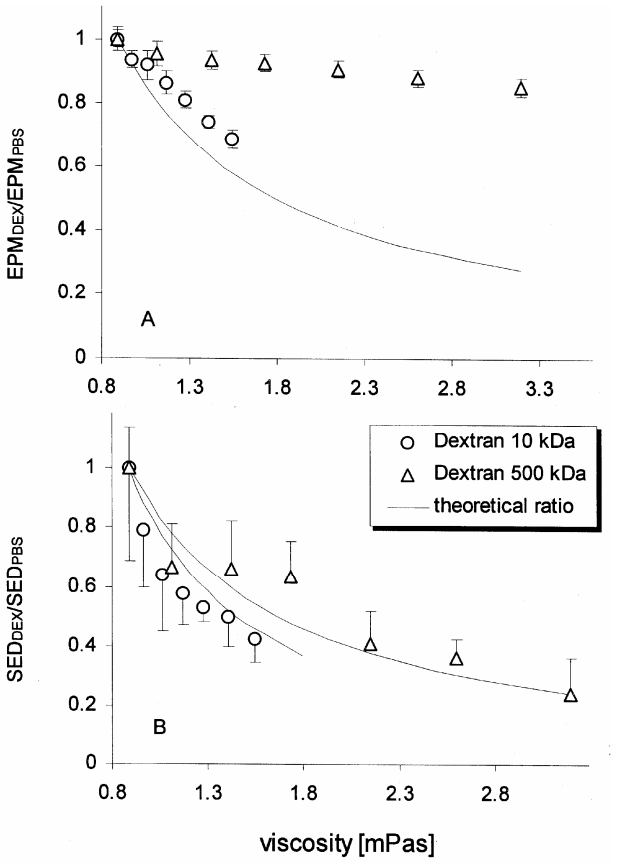

Fig. 4—The relative electrophoretic mobility (EPM) and sedimentation rate (SED) of cells suspended in dextran solutions plotted against medium viscosity. All values are relative to those for cells suspended in dextran-free phosphate buffered saline. The dextrans used had molecular weights of 10 and 500 kDa. Predicted relationships are shown as curved lines; separate lines are shown for the 10 and 500 kDa dextrans predicted sedimentation (SED) behavior due to different increases of medium density caused by these two polymers.

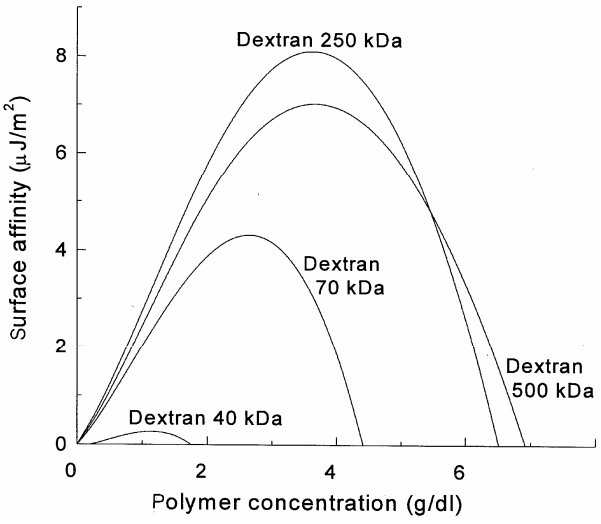

Fig. 5—Predicted surface affinity versus dextran concentration for dextrans having molecular weights of 40, 70, 250, and 500 kDa.

In an extension of the depletion-mediated aggregation model to consider age-separated RBC[49], the calculated results were constrained by: (1) maintaining identical red blood cell EPM values in polymer-free buffer; (2) maintaining the slightly greater EPM for older cells in polymer solutions. Thus, for a given polymer, only glycocalyx thickness or polymer penetration into the glycocalyx served as variables, with both possibilities shown in Fig. 6. These two possibilities are considered below:

Glycocalyx thickness: Considering the effects of decreased glycocalyx thickness δ (Fig. 6A, δ1 *vs* δ2), computed results indicate that a small, 10% decrease (i.e., decrease δ from 5.0 to 4.5 nm) only slightly increases cell EPM by 3-4% yet increases cell affinity by 60% for RBC in a 30 g/l solution of 70 kDa dextran. In turn, this 60% increase in surface affinity can be predicted to yield a two-fold increase in RBC aggregation such as seen for older versus younger cells[11,14].

Glycocalyx penetration: Considering the effects of decreased glycocalyx penetration (Fig. 6B, p1 *vs* p2), computed results indicate that relatively minor changes of glycocalyx physicochemical properties result in marked changes of cell affinity; a 15-20% decrease of polymer penetration results in the same increase of surface affinity and RBC aggregation as seen for the 5.0 to 4.5 nm decrease of glycocalyx thickness[49].

In overview, the model for depletion-mediated aggregation[47] provides a rational framework for explaining the observed equal EPM values in buffer, the different EPM values in polymer solutions, and the marked increases of polymer-induced aggregation for age-separated RBC. That is, compared to less-dense cells, denser cells may have either a slightly thinner glycocalyx or a slightly less polymer penetration into their glycocalyx, and thus greater polymer depletion and larger osmotic forces favoring aggregation[49]. However, the situation is less clear for septic cells, inasmuch as they have equal EPM in buffer and a lower EPM in polymer, thus suggesting less polymer depletion and lower aggregation rather than the higher aggregation observed for RBC from septic animals[7]. One solution to this dilemma is to postulate that cells from septic animals have a slight reduction of surface charge plus an increased interaction of polymer with the glycocalyx[48]. The applicability of the model to other types of RBC (e.g., donor-to-donor variation, enzyme-treated cells, non-human cells) awaits experimental investigation.

Concluding comments

The realization that cell specific factors are important in RBC aggregation became manifest only within the past two decades, and considerable experimental data on the phenomenon have appeared. Although there are now several reports dealing with cellular factors affecting red blood cell aggregation, the field is still in its early stages and thus many questions remain to be answered. In particular, reasons for differences between subjects and between age-separated cells remain unclear. Theoretical models have attempted to develop a mechanistic framework for understanding aggregation, and have increasingly focused on the depletion layer hypothesis rather than the alternative, the cross-bridging hypothesis. However, there are still experimental findings that are currently difficult to explain in terms of the depletion layer hypothesis, and much more

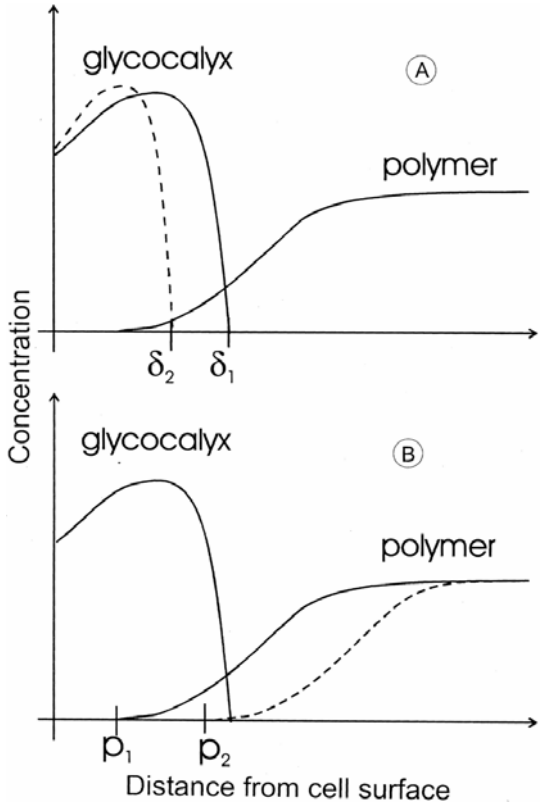

Fig. 6—Schematic representations of two approaches used to predict the effect of altered glycocalyx physicochemical properties on cell-cell affinity. Panel A: differing thickness of the RBC glycocalyx (i.e., δ1 *vs* δ2); Panel B: differing polymer penetration of the glycocalyx (i.e., p1 *vs* p2).

theoretical and experimental work is needed. Further, it is important to stress the potential clinical importance of understanding cellular factors affecting RBC aggregation. Detailed information about such factors should allow the possibility of cellular modification to improve *in vivo* hemodynamics in the rheologically-compromised patient.

Acknowledgement

This study was supported by NIH Research Grants HL15722, HL 70595 and FIRCA IR03 TW01295, and by the Akdeniz University Research Projects Unit. Portions of the material presented were modified from: Rampling, M.W., Meiselman, H.J., Neu, B.H. and Baskurt, O.K., *Biorheology* , 41 (2004) 91.

References

1 Goldsmith H L, Bell D N, Spain S & McIntosh F A, Effect of red blood cells and their aggregates on platelets and white cells in flowing blood, *Biorheology*, 36(1999) 461.

2 Nobis U, Pries A R, Cokelet G R & Gaehtgens P, Radial distribution of white cells during blood flow in small tubes, *Microvasc Res*, 29(1985) 295.

3 Pearson M J & Lipowsky H L, Influence of erythrocyte aggregation on leukocyte margination in postcapillary venues of rat mesentery, *Am J Physiol*, 279(2000) H1460.

4 Bishop J J, Nance P R, Popel A S, Intaglietta M & Johnson P C, Effect of erythrocyte aggregation on velocity profiles in venules, *Am J Physiol*, 280(2001) H222.

5 Bishop J J, Nance P R, Popel A S, Intaglietta M & Johnson P C, Erythrocyte margination and sedimentation in skeletal muscle venules, *Am J Physiol*, 281(2001) H951.

6 Bishop J J, Popel A S, Intaglietta M & Johnson P C, Effects of erythrocyte aggregation and venous network geometry on red blood cell axial migration, *Am J Physiol*, 281(2001) H939.

7 Baskurt O K, Temiz A & Meiselman H J, Red blood cell aggregation in experimental sepsis, *J Lab Clin Med*, 130(1997) 183.

8 Bauersachs R M, Shaw S, Zeidler A & Meiselman H J, Hemorheological findings in uncontrolled type II diabetes mellitus: effects of acute insulin therapy. *Clin. Hemorheol*, 7(1987) 432.

9 Rainer C, Kawanishi D T, Chandraratna P A N, Bauersachs R.M, Reid C.L.,. Rahimtoola S H & Meiselman H J, Changes in blood rheology in patients with stable angina pectoris as a result of coronary artery disease, *Circulation*, 76(1987) 15.

10 Hein H J, Bauersachs R M, Feinstein E I & Meiselman H J, Hemorheological abnormalities in chronic renal failure patients, *Clin Hemorheol*, 7(1987) 430.

11 Meiselman H J, Red blood cell role in RBC aggregation: 1963-1993 and beyond, *Clin. Hemorheol*, 13 (1993) 575.

12 Nordt F J, Haemorheology in cardiovascular diseases: approaches to drug development, *Ann N Y Acad Sci*, 416(1983) 651.

13 Nash G B, Wenby R B, Sowememo-Coker S O & Meiselman H J, Influence of cellular properties on red cell aggregation, *Clin Hemorheol*, 7(1987) 93.

14 Sowemimo-Coker S O, Whittingstall P, Pietsch L, Bauersachs R M, Wenby R B & Meiselman H J, Effects of cellular factors on the aggregation behaviour of human, rat and bovine erythrocytes, *Clin Hemorheol*, 9(1989) 723.

15 Whittingstall P., Toth K, Wenby RB & Meiselman H J, Cellular factors in RBC aggregation: effect of autologous plasma and various polymers, in *Hemorheologie et Aggregation Erythrocytaire*, edited by J F Stoltz (Editions Medicales Internationales, Paris) 1992, 21.

16 Linderkamp O, Ozanne P, Yu P Y K & Meiselman H J, Red blood cell aggregation in preterm and term neonates and adults, *Pediatr Res*, 18, (1984) 1356.

17 Rampling M W, Hughes N & Phillips J, Do red blood cells respond similarly to all aggregants? *Biorheology*, 36(1999) 169.

18 Rampling M W, Whittingstall P, Martin G, Bignall S, Rivers R P, Lissauer T J & Bailey P C, A comparison of the rheologic properties of neonatal and adult blood, *Pediatr. Res*, 25(1989) 457.

19 Whittingstall P & Meiselman H J, Aggregation behavior of neonatal red blood cells, *Clin Hemorheol*, 11(1991) 728.

20 Johnn H, Phipps C, Gascoyne S, Hawkey C & Rampling M W, A comparison of the viscometric properties of blood from a wide range of mammals, *Clin Haemorheol*., 12(1992) 639.

21 Chien S, Usami S, Dellenback R J & Bryant C A, Comparative hemorheology-hematological implications of species differences in blood viscosity, *Biorheology*, 8(1971) 35.

22 Bäumler H, Neu B, Mitlohner R, Georgieva R, Meiselman H J & Kiesewetter H, Electrophoretic and aggregation behavior of bovine, horse and human red blood cells in plasma and in polymer solutions, *Biorheology*, 38(2001) 39.

23 Baskurt O K, Farley R A & Meiselman H J, Erythrocyte aggregation tendency and cellular properties in horse, human, and rat: a comparative study, *Am J Physiol.*, 273(1997) H2604.

24 Rampling M W & Warren O, A comparison of the effects of bromelain digestion on the aggregation of human, equine and bovine red cells, *Biorheology*, 36(1999) 168.

25 Castellini, M, Elsner, R, Baskurt O K, Wenby R B & Meisleman H J Blood rheology of weddell seals and bowhead whales, *Biorheology*, 43(2006) 57.

26 Linderkamp O & Meiselman H J, Geometric, osmotic and membrane mechanical properties of density-separated human red cells, *Blood*, 59(1982) 1121.

27 Ehrly A M, *Therapeutic Hemorheology* (Springer-Verlag, Berlin) 1991.

28 Lowe G D O, *Clinical Blood Rheolog*, (CRC Press, Boca Raton),1988.

29 Stoltz J F, Singh M & Riha P, *Hemorheology in practice* (IOS Press, Amsterdam) (1999).

30 Bauersachs R M, Shaw S J, Zeidler A & Meiselman H J, Red blood cell aggregation and blood viscoelasticity in poorly controlled type 2 diabetes mellitus, *Clin Hemorheol*, 9 (1989) 935.

31 Chong-Martinez B, Buchanan T A, Wenby R B & Meiselman H J, Decreased red blood cell aggregation subsequent to improved glycemic control in type 2 diabetes mellitus, *Diabetic Medicine*, 20(2003) 301.

32 Hacioglu G, Yacin O, Bor-Kucukatay M, Ozkaya G & Baskurt O K, Red blood cell rheological properties in various

rat hypertension models, *Clin Hemorheol Microcirc.*, 26(2002) 27.

33 Kayar E, Mat F, Meiselman H J & Baskurt O K, Red blood cell rheological alteration in a rat model of ischemia-reperfusion injury, *Biorheology*, 38,(2001) 405.

34 Brooks D E, Mechanism of red cell aggregation, in *Blood cells, rheology and aging*, edited by D Platt (Springer-Verlag, Berlin)1988, 158.

35 Chien S, Biophysical behaviour of red cell suspensions, in: *The red blood cell*, edited by D M Surgenor (Academic Press, New York) 1975, 1032.

36 Chien S & Sung L A, Physicochemical basis and clinical implications of red cell aggregation, *Clin Hemorheol*, 7(1987) 71.

37 Snabre P & Mills P, Effect of dextran polymer on glycocalyx structure and cell electrophoretic mobility, C*oll. Polym. Sci*, 263(1985) 494.

38 Lominadze D & Dean W L, Involvement of fibrinogen specific binding in erythrocyte aggregation, *FEBS Lett*, 517(2002) 41.

39 Janzen J & Brooks D E, A critical reevaluation of the nonspecific adsorption of plasma proteins and dextrans to erythrocytes and the role of these in rouleaux formation, in *Interfacial phenomena in biological systems*, edited by M. Bender (Marcel Dekker, New York) 1991,193.

40 Armstrong J K, Meiselman H J & Fisher T C, Evidence against macromolecular bridging as the mechanism of red blood cell aggregation induced by nonionic polymers, *Biorheology*, 36(1999) 433.

41 de Gennes P G, Model polymers at interfaces, in: *Physical basis of cell-cell adhesion*, edited by P. Bongrand (CRC Press, Boca Raton, FL) 1988, 39.

42 van Oss C J, Arnold K & Coakley W T, Depletion flocculation and depletion stabilization of erythrocytes, *Cell Biophys*, 17(1990) 1.

43 Bäumler H, Donath E, Krabi A, Knippel W, Budde A & Kiesewetter H, Electrophoresis of human red blood cells and platelets. Evidence for depletion of dextran, *Biorheology*, 33(1996) 333.

44 Bäumler H, Neu B, Donath E & Kiesewetter H, Basic phenomena of red blood cell rouleaux formation, *Biorheology*, 36(1999) 439.

45 Arnold K, Zschoering O, Barthel D & Herold W, Exclusion of poly(ethylene glycol) from liposome surfaces, *Biochim. Biophys. Acta*, 1022(1990) 303.

46 Neu B & Meiselman H J, Sedimentation and electrophoretic mobility behavior of human red blood cells in various dextran solutions, *Langmuir*, 17(2001) 7973.

47 Neu B & Meiselman H J, Depletion-mediated red blood cell aggregation in polymer solutions, *Biophys J*, 83(2002) 2482.

48 Baskurt O K, Tugral E, Neu B & Meiselman H J, Particle electrophoresis as a tool to understand the aggregation behavior of red blood cells, *Electrophoresis*, 23(2002) 2103.

49 Neu B, Sowemimo-Coker S O & Meiselman H J, Cell-cell affinity of senescent human erythrocytes, *Biophys J*, 85 (2003) 75.