170c Blood Cell Separation Issues in Miniature Blood Diagnostic Kits

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Application of routine blood tests in near-patient testing (NPT) has seen a great deal of interest recently, especially in coagulation test of bleeding susceptibility of patients undergoing surgery and monitoring of patients undergoing anti-coagulation therapy for the prevention of blood clots. However, most of the current blood tests are expensive in terms of reagents or equipment needs and require close scrutiny and large blood volume to obtain accurate results. As frequent monitoring is expected especially during some oral drug dose therapy (Heparin), blood sampling in a home environment is generally limited to the 'fingerstick' method, which produces relatively small volumes of whole blood, generally from 5 to 30 microliter.

Microfabricated lab-on-chip devices constituted with network of micro-channels and embedded micron or nano electrodes is a promising alternative method for some blood tests. State-of-the-art lithography techniques allow the manufacture of high-quality micro-array electrode and measurement of certain electrical signals have been used previously to study whole-blood clotting times, clot formation and reported as an effective way for miniature kits detection. But other tests with small amount of whole blood from finger-stick remain unsatisfactory due to several micro-fluidic particle aggregation and separation issues. They include failure of blood suspension to penetrate a capillary with radius R less than 50 microns due to blood cell aggregation even if the capillary is perfectly wettable for loading and the necessity of separating blood cells from plasma if the former interfere with the specific blood test like coagulation and glucose detection.

In this report, detailed study on capillary wetting for blood suspensions through glass capillaries and accordingly adjustment on the wetting properties of capillary inside wall, deformability of erythrocyte was found to responsible for the different wetting dynamics of red blood cell (RBC) suspensions during their invasion into capillaries. Normal RBC suspensions failed to penetrate more than 1cm into a glass capillary when the capillary radius is smaller than a critical value that is dependent on the erythrocyte concentration (about 50 micron for whole blood). In contrast, suspensions of rigidified RBCs, after cross-linking with different concentrations of glutaraldehyde or incubating with 100ng/ml of an endotoxin, could penetrate any capillary that is larger than the erythrocyte dimension. The effect of RBC deformability on penetration was attributed to the enhanced shear-induced migration of normal deformable RBCs toward the capillary centreline, which imparted a higher average velocity to the RBCs than the average plasma velocity. As a result, the erythrocytes advanced into the capillary faster than the wetting meniscus, and packed behind it to form a concentrated slug. This tightly packed slug had a high hydrodynamic resistance that could arrest the penetrating flow of concentrated suspensions into the small capillaries. As RBC of sepsis, sickle cell of anaemia and leukaemia patients are known to be more rigid, a diagnostic kit for these diseases has been designed based on the wetted length and wetting time, as both are sensitive signatures of RBC rigidity.

Realizing the penetration failure for normal blood suspension is due to radial migration by shear gradient, a strategy for countering this inward migration is developed. The sedimentation velocity of the RBC is independent of the shear rate and hence at sufficiently low penetration speeds, its magnitude must exceed the shear-rate dependent migration velocity. A critical resuspension velocity is derived below which the RBC will settle to the bottom half of the capillary. For whole blood, this critical penetration velocity is about 100 microns/s for a 100 micron capillary. With sedimentation, a clear plasma slug actually develops behind the meniscus, in contrast to the concentrated slug for penetration failure conditions above the resuspension velocity. Penetration experiments were carried out with raw capillaries and prewetted ones, the latter were washed by a Bovine Serum solution (diluted 1:10 in PBS) to endow a meniscus velocity lower than the critical resuspension velocity. The meniscus and the clear

plasma slug penetrate successfully to an embedded electrode sensor array where an impedance spectrum analysis is used to determine the conductance and capacitance of the RBC-free plasma during coagulation. The impedance spectra for blood samples with different coagulation rates are shown to be very different at a specific frequency corresponding to the inverse RC time of the fibrin network that forms during coagulation. We have hence developed a simple design strategy for capillary blood diagnostic kits. Such easily fabricated kits do not contain any micropumps or micro-vales and rely only on wetting to load the blood sample. Yet, with careful analysis of the suspension rheology, the simple capillary kits can be designed to detect the deformability of RBC and to separate RBC from plasma for blood coagulation tests and a host of other blood diagnostic tests.