Correlative Microscopy in Life and Materials Science

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Scanning electron microscopy observation of erythrocyte ghosts isolated from slaughterhouse blood by gradual hemolysis

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Slaughterhouse blood, although usually treated as waste and discarded, represents an inexpensive source of red blood cell membranes (ghosts). Detailed chacterization of ghosts and use of advanced biotechnological tools for their modifications, could enormously contribute to its application for delivery of active compounds and for manufacturing of complex delivery systems (e.g. multilayer microcapsules with the core of ghosts).

In this work, isolation of erythrocyte ghosts from bovine slaughterhouse blood is based on gradual hypotonic hemolysis [1]. The obtained bovine ghosts were analysed from the aspect of morphology and structural integrity by means of flow cytometry, phase-contrast and scanning electron microscopy.

Flow cytometric analysis was performed on CyFlow® SL flow cytometer (Partec, Münster, Germany) using FlowMax 2.4 software (Partec, Münster, Germany). The samples for scanning electron microscopy were prepared as follows: Packed erythrocytes and ghosts were fixed in 2.5 % glutaraldehyde solution in PBS for 1h and washed twice in PBS. The preparations were post-fixed in 2 % osmium tetroxide for 1 h, rinsed in PBS, dehydrated through a graded ethanol series (10, 30, 50, 70, 95 and 100 %) for ten minutes each, and then subjected to critical point drying using liquid carbon dioxide (Bal-Tec CPD030 Critical Point Dryer). After gold coating, samples were visualised on a field emission scanning electron microscope (FE-SEM), a TESCAN MIRA 3 XMU, operated at 10 kV.

The morphological changes of erythrocytes and appearance of erythrocyte ghosts after gradual hemolysis and restoration of isotonicity, were assessed by phase contrast microscopy using Olympus CKX 41 inverted microscope (Olympus Europa Holding GmbH, Hamburg, Germany). (Figure 1.) The micrographs showed intact cellular structures of ghosts, having altered cellular content (without the content of hemoglobin), and approximately the same size of ghosts and starting erythrocytes. The assessment of erythrocytes and ghosts size was performed by forward scatter (FSC) analysis on flow cytometer. As presented in Figure 2a, two populations of erythrocytes are visible (populations R1 and R2), while the same analysis of ghosts (Figure 2b) revealed another population of small “events” (population R5). We assumed that fragmentation of ghosts was induced by shear stresses associated to flow cytometric measurements. The presence of small vesicles (R5) was not detected under phase contrast microscope. Detailed insight into ghosts morphology obtained by FE-SEM (Figure 3b), showed slightly distortion from erythrocyte shape (Figure 3a), an altered surface texture with increased bilayer curvature and existence of numerous invaginations. This kind of morphology has been reported for human erythrocytes when hypotonic PBS buffer was used in a production of ghosts [2]. Scanning electron micrographs have indicated that there is no difference in size between red blood cells and ghosts obtained by gradual hemolysis. The morphological alterations of bovine ghosts observed by FE-SEM, may be related to the changes of transbilayer lipid asymmetry or cytoskeleton content (as reported for human ghosts [2]). They probably triggered formation of small vesicles during flow cytometry analysis.

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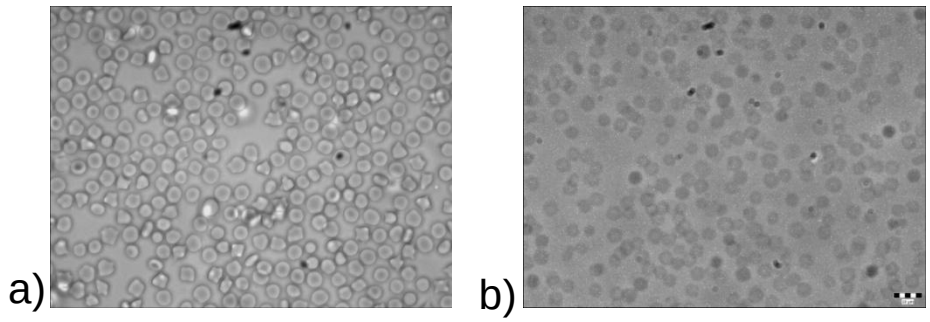

Figure 1. Phase contrast micrographs of erythrocytes from bovine slaughterhouse blood (a) and erythrocyte ghosts obtained by gradual hypotonic hemolysis (b); Magnification ×400.

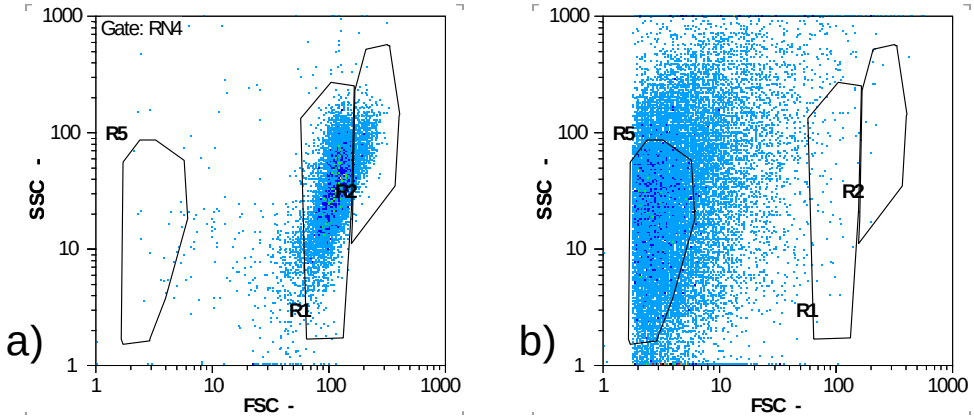

Figure 2. Flow cytometric analysis of bovine erythrocytes (a) and ghosts obtained by gradual hemolysis (b); R1 and R2 - erythrocyte populations, R5 - small vesicles.

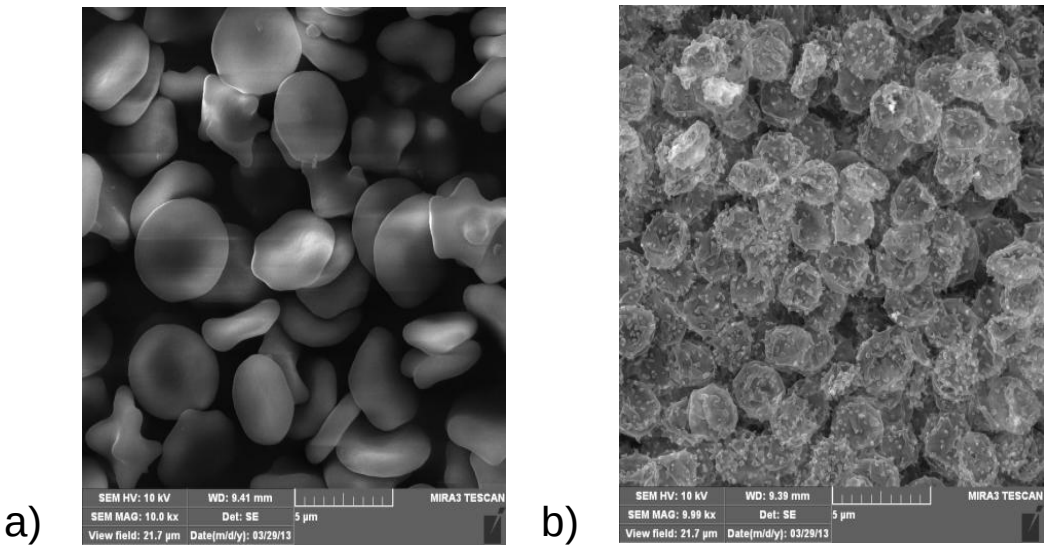

Figure 3. Scanning electron micrographs of bovine erythrocytes (a) and ghosts obtained by gradual hemolysis (b).