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Phase of Division and Budding of Human Erythrocytes in Serum-Free Cultures and Further Refinement Mass Production Plan by Amphiphilic Surfactants

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The presence of erythropoietin receptors and erythropoiesis on erythrocytes is disputable. However, the specific binding of erythropoietin to mammalian erythrocytes was reported [1-6] as well as gene expressions [7-9]. Recently, we proposed a growth-division and fission process involving adult human erythrocytes [10]. More recently, Andes-Koback and Keating [11] and Terasawa et al. [12] reported the budding and division of primitive anucleate model cells. Here, we show the division and budding of human erythrocytes following the delayed addition of erythropoietin in serum-free cultures and further refinement mass production plan by amphiphilic surfactants [13-15].

Normal adult human erythrocytes were isolated from the blood of the authors collected in EDTA-Na_2 tubes by venipuncture. Erythrocytes were separated by centrifugation at 400 g for 10 minutes. A total of 50 µl of packed red blood cells was suspended in 10 ml of DMEM/F12, and then cultured in disposable conical tubes, then examined on days 0, 7, 14, 21, 28, 35 and 42 of culture at room temperature.

On days 0, 7, 14, 21, 28, 35 and 42 of culture, each red blood cell suspension (50 µl) was individually lifted from the primary cultures, and then treated with an equal or double the volume of DMEM/F12 supplemented with 5 units/ml of human erythropoietin in plastic microtubes at room temperature. Each days of culture, 0.5 µl of the red blood cell/5U erythropoietin mixture was dropped on glass cover slips and the cover-slips were inverted over a hole in micro slide glasses, sealed with chemical glue (hanging-drop preparations), and observed by light microscopy (Figure 1).

Further refinement mass production plan by amphiphilic surfactants (boldface).

- Manmalian blood, 400 g, 10 min
- Packed red cells, 50 µl + DMEM/F12, 10 ml. Culture (1 day~5 weeks)

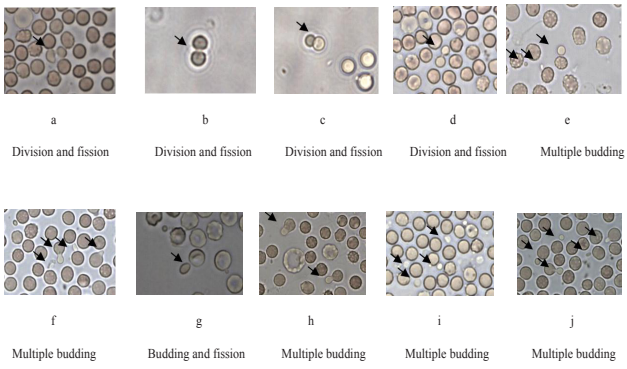

Figure 1: Light microscopic photographs of various phases of division and budding of human erythrocytes (arrows) Objective lens ×60.

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- Red blood cell suspension, 50 µl (3.0×10^8 cells/ml) + amphiphilic surfactants (0.05 % poloxamer 188 [13,14], or 0.05 % polysorbate 80, or 50 µM sodium dodecyl sulfate [15], etc.), 10~50 µl mixture (30~120 min)

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- Red blood cell suspension, 50 µl + erythropoietin (5 units), 50 µl Culture (1~8 h)

↓

- Hanging drop culture, Red blood cell and erythropoietin suspension, 0.5 µl. (2 h~3 days)

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- Fission [10-12,15] (10 min~6 h)

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