

9th World Summit on Neonatal Nursing and Health Care
49th International conference on Prosthodontics & Restorative Dentistry

November 17, 2023 | Webinar

Erythrocyte micro vesiculations in health and diseases

Ugochukwu Obinna Maluze
Torrens University, Australia

Aim: To demonstrate (using Guava Easy flow-cytometer) that micro vesicles are released from erythrocyte membrane naturally in normal and disease conditions, without inducing with calcium chloride; and also, to know how many erythrocytes makes a micro vesicle.

Methods: 15mls of blood samples from the stored in CPDA or SAG-M was provided by NHS blood and transplant, and 10 ml of this blood were mixed thoroughly with 30ml of phosphate buffered saline in a 50ml centrifuge tube, and centrifuge immediately at 600 x g at 4 degrees centigrade for 10 minutes to remove soluble plasma proteins. The supernatant was removed and discarded after centrifugation. This process was repeated three times in order to remove any erythrocyte bound plasma proteins. Erythrocytes were then counted with haemocytometer using x10 objective lenses and x40 for magnification). The number of red blood cells countered were recorded and documented. Following washing, the cells are re-suspended in 10ml of RPMI 1640 (to bath the cells) and incubated at 37 degrees centigrade in shaking water bath for 45mins, after which the samples were placed in ice to stop the reaction. The samples were centrifuge at a low speed of 160 x g at 4 degree for 15 minutes to pellet the erythrocytes. The eMV containing the supernatant was transferred to a fresh tube and centrifuge at a higher speed of 4000 x g for 60 minutes at 4 degrees centigrade to remove the cell debris and ghost cells, after which the supernatants were transfer to another fresh tube and sonicate in a sonicating water bath for 5 minutes at 4 degrees centigrade prior to centrifugation to disperse aggregated exosomes. The supernatant was further centrifuge and discarded and the remaining eMV pellet was re-suspended in 100ul of phosphate buffered saline for immediate analysis. Flow cytometry analysis was conducted using 10ul of the eMV stock sample which were diluted in 190ul (1:20) of phosphate buffer saline. Biuret protein

assay and Agarose gel electrophoresis were performed. Haemolysed red blood cell was used as a negative control method.

Results: Erythrocyte cell counts was performed using haematology cell counting chamber and analysed with Guava flow cytometer on three consecutive times. The number of cells counted was greater than 400cells. About 3446666 million of micro vesicles were released by 1ml of the isolated erythrocyte. The unknown protein component of micro vesicles was determined as 2.5mg/ml and the protein bands separation were identified using agarose gel electrophoresis.

Conclusion: The isolated micro vesicles were analysed using Guava Easy flow cytometer. The protein concentration was quantified using Biuret assay method, and the protein band separation was analysed using Agarose gel electrophoresis. Knowledge of good laboratory practices, clinical procedures and Knowledge of Laboratory safety and infection control procedures including standard precautions and hazardous chemical handling were very useful in the laboratory aspect of this report.

Biography

Ugochukwu Obinna Maluze is an ambitious, dedicated, diligent, self-motivated and hardworking young man with a good career excellence. He had qualifications on bachelor's degree on Medical Laboratory Science (Ebonyi State University Nigeria), and postgraduate qualifications on Biomedical Science (London Metropolitan University, UK), Biomedical Engineering (University of Bedfordshire, UK), Public Health Advanced (Torrens University Australia) and a course on Clinical toxicology (London Metropolitan University, UK). He has published two articles' journals on "Erythrocyte micro vesiculations in health and diseases" and "Risk factors affecting the prevalence of breast cancer among females aged 40-69 years in Australia.

maluzeugo2020@gmail.com

Received Date: September 13, 2023; **Accepted Date:** September 15, 2023; **Published Date:** December 26, 2023