The different types of cancer cells lines

Exosomes are nanoscale, extracellular vesicles secreted by most of the cells [1] and differ in their size, shape and molecular composition from other vesicles released from the cell [2]. Exosomes have a round to cup shaped morphology with a lipid bilayer membrane and released into the extracellular spaces and the size of exosomes varies from 30nm to 150nm [3,4]. Besides their nano size morphology other defining characteristics of exosomes include density gradient of 1.13-1.21g/ml [5] and protein markers such as the tetraspanins (CD9, CD63, CD81), Alix, Hsp70, Tsg101 [6]. It has been established that cells can release various types of extracellular vesicles such as exosomes, ectosomes, micro-vesicles and recently identified large oncosome [7] therefore, it is very important to characterise exosomes prior to any exosomal investigation [8]. There are several methods available to identify and characterise exosomes including electron microscopy (EM), atomic force microscopy (AFM), nano particle tracking analysis (NTA), Dynamic light scattering (DLS), western blot and flow cytometry.

In the past few years, there have been huge interest on exosomes due to their use as a biomarker tool for cancer research.[10]. Several studies have suggested the existence of exosomes with distinct composition and biological information in the same population, which have different effects on recipient cells [2,11]. In recent years, proteins of tumour derived exosomes have been investigated by mass spectrometry based proteomics to search for potential biomarkers [12]. For example, in a study on melanoma patients, the expression of CD63+, a tetraspanin super family member shown significant increase compared to healthy control [13]. In another study, one more member of the tetraspanin protein family CD81 was found elevated in their expression in chronic hepatitis C patients [14]. In any proteomic study, protein concentration of the sample may be crucial especially if the experiment is based on gel electrophoresis and the amount of exosomal protein is correlated with the exosome number [15] but knowing the total amount of proteins is sufficient for most of the times, but it has been reported that some applications require the exact number of exosome in the solution such as the use of exosome as a vehicle for drug delivery as it is essential to standardise the number and size of exosomes for accurate doses of drug delivery. Besides being a new tool for biomarker research, exosomes have also been exploited as a delivery vehicle for therapeutic agents, due to their stability, ability to cross blood brain barrier (BBB) and availability in most body fluids. The number of exosomes is important as it helps to identify the relationships with the parent cell of the exosome, state of the cells and to understand the exosome signals [16]. For example, increased number of exosome and their miRNA cargo was observed in an alcoholic hepatitis when tested on a mice model fed with alcohol.

Cells release exosomes in the biological fluids during their growth phase to perform the

biological function such as transferring genetic materials and cell to cell communication, immune response, removing unnecessary materials out of the cell [18]. These exosomes are taken up by other cells from the microenvironment [19]. The time exosomes stays in the biological fluid varies between cell types, for example, the half-life of the exosomes from B16 melanoma cells is 30 minutes and this was observed when exosomes were labelled with fluorescent dye to check their stability [20]. Whereas, exosomes from human platelet concentrate showed a half-life of 5.5 hours [21]. Within these short period of times, exosome caries various proteins and RNAs depending on the cell of origin.

The aim of this study was to isolate and characterise exosomes from three different types of cancer cells lines, the lung cancer cell line H358, leukemic cell line THP1 and breast cancer cell line MCF7, quantify the number of exosomes and exosomal protein pattern during their cellular growth.