Supporting Information

for

Optimal Conditions for Labelling of 3T3 Fibroblasts with Magneto-liposomes without Affecting Cellular Viabilities

Stefaan J. H. Soenen, Johan Baert, and Marcel De Cuyper*

Designation of the cationic surfactant content in vesicles and MLs: In small sonicated vesicles with a diameter of about 25-30 nm nearly one-third of the phospholipids are in the inner leaflet and \( \frac{1}{3} \) in the outer.\[^1\] Upon incubation with an equimolar amount of neutral MLs, only the cationic lipids present in the outer leaflet of the vesicles are able to transfer as thermodynamically highly unfavourable transbilayer movements from the inner to the outer layer (and vice versa) are not thought to occur within the time span of the experiment.\[^2\] Thus, ultimately, only \( \frac{1}{3} \) of the cationic lipids originally present in the vesicles will show up in the outer layer of the ML coat. For instance, by incubating DMPC-DOTAP (50/50; molar ratio) vesicles with an equimolar amount of DMPC MLs, at equilibrium, vesicles are produced with 33% of the original amount of DOTAP in the outer bilayer shell of both the vesicle and ML population, and the remaining 33% DOTAP remains entirely in the inner shell of the vesicle bilayer. Or, if one relates these percentages to the total amount of lipids present in the entire bilayer of the particles (i.e. also taking DMPC into account), this means that at equilibrium the DOTAP content in the outer leaflet of vesicles and MLs equals 16.66%. Thus, vesicles with an overall DOTAP content of 25% (16.66% DOTAP in the outer and 8.34% DOTAP in the inner layer) present a similar surface as MLs, with a DOTAP content of 16.66% (DOTAP is solely present in the outer layer). Therefore,
we considered vesicles and MLs with the above-mentioned composition as being comparable. Throughout this work we have designated these structures as 16.66% DOTAP-containing vesicles and 16.66% DOTAP-containing MLs.

![Figure S1](image-url): Intracellular iron content at an incubation time of 24 hours of 3T3 fibroblasts incubated with neutral (100% DMPC); cationic (DOTAP-DMPC) MLs (16.66% DOTAP) or anionic (DMPG-DMPC) MLs (10% DMPG). The reference cells are control cells not incubated with MLs, but otherwise treated identically. The error bars indicated are mean ± SEM (n = 10).

Iron uptake per cell: The combined results of the intracellular iron determination together and the BCA protein assay make it possible to determine the average amount of iron taken up per cell. To do so, the results of the BCA assay must be transformed to convert a certain amount of protein into a certain number of cells. Meisler showed that the amount of protein per cell is not constant for 3T3 fibroblasts and that the amount is inversely proportional – within limits – to the density at which the cells are cultured.[3] When the 3T3 fibroblasts in the Falcon flasks at 80% confluency were Trypan blue-stained and counted in a Bürker chamber, we obtained, on average, about 8 million cells per culture, or 8 million cells per 75 cm², which gives a density of a little over 1.10^5 cells per square centimetre. According to the data presented by Meisler, at this density, 10^6 cells is equivalent to a theoretical value of ca. 200 µg of protein.
In order to obtain a more precise number, we performed the BCA assay on a known number of cells, resulting in more accurate protein content per cell. Cells were counted in a Bürker chamber, after which the cell suspension was centrifuged (1500 rpm for 10 min; Megafuge 1.0, Heraeus, Kendro Laboratories, Germany) and the supernatant removed, leaving the cell pellet, which was resuspended in lysis buffer at a concentration of 5.10^4 cells per 25 µL of lysis buffer. Subsequently, 50 wells were filled with 25 µL of cell-containing buffer and the BCA assay performed as described above. The average absorbance at 570 nm for the 50 wells was 0.3364. The standard curve showed that the absorbance was related to the concentration by a second order polynomial curve, \( y = -2\times10^{-7}x^2 + 0.001x - 0.0145 \), where \( x \) is the absorbance and \( y \) the protein concentration, with an \( r^2 \) value of 0.9994. Using the standard curve, our absorbance of 0.3364 was equivalent to a protein concentration of 379.75 µg/mL. Thus, for 25 µL of cell suspension used, this equals to 9.49 µg of protein, representing 5.10^4 cells. For 10^6 cells, this corresponds to 189.87 µg of protein, which is near the theoretical value of 200 µg found by Meisler,\(^3\) indicating that our results were trustworthy and that, in our hands, the BCA assay was a safe and accurate method for determining the total number of cells.

References

Colour versions of the main figures:

Scheme 1.
Figure 1.
Figure 3.
Figure 7.