**P6 — Micromanipulation of Lipid Vesicles and Cells in Drug Delivery Using Optical Tweezers**

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**Abstract**

In photodynamic therapy (PDT) several photosensitisers (PS) are hydrophobic compounds. To improve the anticancer behaviour of PS, liposomes or polymeric nanoparticles and low-density lipoproteins are currently used as drug delivery systems in the treatment of malignant tumours. These nanoparticles with encapsulated dyes could be multifunctional bio-photonic systems that serve as non-invasive tumour diagnosis and therapy systems with photodynamic properties. Optical tweezers and laser ablation can be used to manipulate the liposomes and to penetrate nanoparticles and cell walls or to overcome the cell wall to perform cell fusion. In this work we describe preliminary results obtained for selective optical manipulation and fusion of the photosensitized nanoparticles. We optimize the protocols for achieving single cell - lipid vesicle contact in view of delivering the cytostatic drugs into malignant cells. For that purpose, Giant Unilamellar Vesicles (GUVs) were prepared and Trypan Blue dye was incorporated into lipid vesicles. As malignant cells B16F10 - murine melanoma cells were used. The individual cell-vesicle interaction was studied by following up the behaviour of the cell-vesicle pair put in contact by the aid of optical tweezers.

As it has been reported, short-chain alcohols affects monolayer bending and promote hemifusion between fluorescent stained liposomes. So in our experiments the liposomes sample was immersed in methanol solution in order to initiate hemifusion between the trapped liposome bilayers, which have come in contact.

**Materials and methods**

Liposome-cell preparation:

1. Liposomes of egg yolk phosphatidylcholine (Lipoid Gmbh) were prepared in aqueous buffer solution (HPLC grade H₂O) by hydration for 1 hour (30 mg/ml) and were stained with the hydrophilic dye Methylene Blue (MB), with a concentration of 4 µM. Methanol (Merck KGaA) of 10% v/v concentration was added in the stained liposomes samples.
2. B16F10 - murine melanoma cells were used. They were cultured in 3 cm Petri dishes, at 37°C, 5% CO2, in Dulbecco’s MEM (Gibco) supplemented with 10% foetal calf serum (Gibco), 1% of antibiotics (BioClone).

3. Giant Unilamellar Vesicles (GUV) were prepared in rotaevaporator device from 1,2-Dioleoyl-sn-glycero-3-phosphocholine (lyophilized powder).

4. Trypan blue dye was incorporated into lipid vesicles during their preparation protocol, in order to make them visible under microscope. Also we used Dextran 70 fractions in order to obtain GUVs with a refractive index higher than that of the suspending medium.

Optical set up:
1. Optical trapping of GUVs was done by using a diode laser at a wavelength of 808 nm
2. The laser was reflected by a dichroic mirror and focused by using an inverted Leica DMIL microscope with a 100X/1.25 oil-immersion objective.

Conclusions
Trapping GUVs requires loading them during preparation with a medium having a higher refractive index than that of the suspending solution.
Osmolarity of the GUV medium play a key role in preserving viability of the cell in suspension.
A sequence of fusion events was observed between the trapped multilammelar liposomes which leads to the formation of a large in volume multilammelar vesicle which remain trapped in the laser focus.
Methanol promotes liposome hemifusion by reducing the bending rigidity of the bilayers and facilitating the transient breakage of the continuity of each of the contacting mono-layers. To overcome this energy, the breaking continuity barrier can be facilitated at close enough distances between membranes. This was achieved by the optical trapping of the liposomes. Complete fusion takes place almost instantaneously. Further experiments using fusogenic agents as well as applying electrical pulses will reveal, the least invasive ways for GUV – cell fusion.
ABSTRACT
Some photosensitizers (PS) used in PDT are hydrophobic compounds. This impedes their penetration into the cytosol which is hydrophilic. To improve the delivery of PS to malignant cells, liposomes or polymeric particles and low-density lipoproteins are used as drug delivery systems in tumour therapy. These particles, when encapsulated or covalently linked to the therapeutic drug, can be used as multifunctional bio-photonic systems that serve as non-invasive tumour diagnostic and therapeutic tools. The combined photodynamic and therapeutic effects can be achieved by the liposome-mediated delivery of PS and pro-drug(s). In this work, we describe preliminary results obtained for selective optical manipulation and fusion of the cell/vesicle walls to perform the fusion. In this work, we describe preliminary results obtained for selective optical manipulation and fusion of the cell/vesicle walls to perform the fusion. In this work, we describe preliminary results obtained for selective optical manipulation and fusion of the cell/vesicle walls to perform the fusion.

MATERIALS AND METHODS
Liposome cell preparation:
1. Liposomes of egg yolk phosphatidylcholine (Lipoid GmbH) were prepared in insulin buffer solution (HPLC grade H2O) by hydration for 1 hour (20 mg/ml) and were stained with the hydrophobic dye Methylene Blue (MB) at a concentration of 4 μM. Methanol (Merek XGAS) of 3% v/v concentration was added in the stained liposomes samples.
2. Human breast cancer cells were used. They were cultivated in 3 cm Petri dishes, at 37°C, 5% CO2, in Dulbecco’s MEM (Gibco) supplemented with 3% fetal calf serum (Gibco), 1% of antibiotics (Biotone).
3. Giant Unilamellar Vesicles (GUVs) were prepared in a recrystallization device by a Dicsoxoyl-glycerol-3-phosphocholine (DSPC) lipid.
4. Trypan blue dye was incorporated into liposomes during their preparation protocol, in order to make them visible under microscope.

RESULTS AND CONCLUSIONS
A sequence of fusion events was observed between the trapped multilamellar liposomes which lead to the formation of a large multilamellar vesicle which remains trapped in the laser focus. Under described experimental conditions, but maintaining the membranes of GUVs and cell in close contact for a maximum 2 minutes leads to a burst fusion. The mechanism proposes liposome burstiness by reducing the binding energy of the bilayers and facilitating the transmembrane breakdown in conformity with each of the monolayers in contact. This may occur as seen random breakages in unilamellar membranes which was achieved by optical trapping of the liposomes.

References: