Characterization and Optimization of Nanoliposomes to Deliver 17β-Estradiol

Kristen Bowey[1], Isabelle Cloutier[3], Jean-François Tanguay[3], Maryam Tabrizian[1,2].
[1]Biomedical Engineering Department and [2]Faculty of Dentistry, McGill University, Montréal, Canada;
[3]Department of Medicine, Montréal Heart Institute, Montréal, Canada

Statement of Purpose: Despite extensive applications of small hydrophobic pharmaceutical agents, such as 17β-estradiol (E2), efficient delivery is often challenged by short biological half-lives and rapid clearance in the body. Since high doses must be administered to compensate, toxicity and undesirable side effects may be an obstacle to effective treatment regimes. An alternative to a bolus delivery is incorporation into liposomes. We hypothesize that encapsulation of E2 within nanoliposomes can aid to modulate therapeutic delivery E2. In this study, we investigated the preparation and characterization of liposomes for E2 delivery to the myocardium. **Methods:** Nanoliposomes were prepared by thin film hydration. The main lipid, egg phosphatidylcholine (EPC), 1, 2-dialmitoyl-sn-glycero-3-phosphocholine (DPPC), or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), didecyldimethylammonium bromide, cholesterol, and E2 were dissolved in chloroform:methanol (4:1). Solvent was removed by rotary evaporation, rehydrated with buffer for 30 min under constant rotation (60 rpm), and extruded through membrane filters (0.4, 0.2 μm) with an Avanti® Mini-Extruder (Avanti Polar Lipids, USA). **Encapsulation Efficiency** After un-entrapped E2 was removed by tangential flow filtration (TFF), the E2 EE and loading capacity (LC) was determined by drying liposomes overnight and dissolving in methanol to solubilize the formulation. The sample was then assayed by HPLC. **Release Kinetics** Known quantities of liposomes were placed into a Float-A-Lyzer® G2 device (Spectra/Por, molecular weight cutoff of 3.5-5 kDa, Spectrum Laboratories Inc. USA) and release was measured over 48 h. Formulations were dialyzed against 40% ethanol in phosphate buffered saline (pH=7.4). All release studies were conducted at 37°C under gentle agitation. At appropriate time intervals, aliquots were removed and E2 was assayed by HPLC. **Cytotoxicity** The effects of free and encapsulated E2 on cardiomyocyte viability was assayed by MTT (Invitrogen, USA). Varying concentrations of free and encapsulated E2 were incubated on cells plated at a density of 40,000 cell/cm^2, for 24 h. **Results:** The composition of the liposomal core was investigated to maximize the LC of E2 within the lipid bilayers. **Table 1** shows that E2 LC was influenced by the main lipid composition. E2 was incorporated into liposomes composed of phospholipids in the order of DOPC>EPC>DPPC for equal levels of E2 loading. The lowest LC was observed in DPPC, which may be because DPPC is a saturated lipid that tends to form a more rigid membrane structure compared to DOPC and EPC, which are primarily composed of unsaturated phospholipids. The highest LC with the least amount of E2 loss (i.e. highest EE) was achieved in the formulation of DOPC, with an initial E2 loading of 5% mol/mol, which was used herein.

Table 1. *Effects of main lipid and initial E2 loading on encapsulation efficiency and loading capacity.*

Main Lipid	*E2 Loading (%mol/mol)*[1]	*EE (%)*	*LC (μg/mg)*[2]
EPC	10	18.9 ± 0.7	5.5 ± 0.3
	5	41.7 ± 2.2	5.8 ± 0.3
	1	56.0 ± 3.4	1.9 ± 0.1
DOPC	10	25.1 ± 1.6	7.1 ± 0.5
	5	56.2 ± 4.1	7.8 ± 0.6
	1	68.6 ± 2.7	1.9 ± 0.1
DPPC	10	8.6 ± 0.4	2.7 ± 0.1
	5	15.6 ± 0.5	2.3 ± 0.1
	1	65.0 ± 8.3	2.3 ± 0.3

[1]*E2 loading was calculated based on the molar ratio of the main lipid*
[2]*Loading capacity of E2 is based on total nanoparticulate weight*

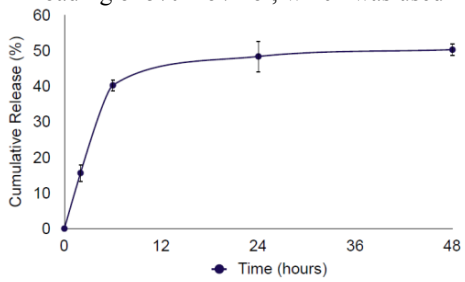

Figure 1. *E2 in vitro release kinetics from liposomes*

Release kinetics showed a quick initial release within the first 6 h, followed by a more sustained release that reaches a plateau around 50% release.

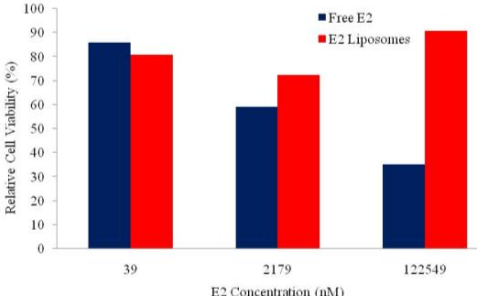

Figure 2. *Cytotoxicity study of free and encapsulated E2*

As demonstrated in **Figure 2**, liposomes produced no adverse toxic effects on cardiomyocytes, even conferring a benefit at higher levels of E2. **Conclusions:** Results from this study showed that E2 was incorporated to the greatest extent in nanoliposomes composed of DOPC, resulting in an EE of ~ 56% and an LC of ~8 μg E2 /mg of liposomes. Under *in vitro* conditions, roughly 50% of the E2 was released and no cytotoxicity effects were observed. The next step in this study will investigate the application of biopolymers on the surface to alter release kinetics and investigate the effects on induced ischemic-reperfusion injuries both *in vitro* and *in vivo*. **Acknowledgments**: NSERC Discovery Grant/CIHR Regenerative Medicine/Nanomedicine Grant.