Niosomal photodynamic therapy as an *ex vivo* strategy to eradicate malignant cells from tissue

Saeid Moghassemi[1], Arezoo Dadashzadeh[1], Christiani A. Amorim[1]

[1]Pôle de Recherche en Gynécologie, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, 1200 Brussels, Belgium

Background: One of the most interesting applications of photodynamic therapy (PDT) is *in situ* and *ex vivo* purging of various tissues from malignant cells [1, 2]. In this regard, the self-aggregation tendency and poor bioavailability of common photosensitizers (PS) are important disadvantages, which can be overcome by the niosomal formulation of PSs. This approach improve PS penetration and bioavailability in the tissue in *ex vivo* studies [3, 4]. The aim of this research is therefore to prepare a niosomal PS formulation, as a third-generation PS, and to analyze (i) *ex vivo* PDT toxicity for tissue fragment, (ii) *in vitro* PDT efficiency, and (iii) single-cell morphology after ORN-based PDT.

Methods: Blank (BLK) and OR141-loaded niosomes (ORN) were prepared by a reversed-phase evaporation method (Fig. 1) [5]. After ORN fluorescence microscopy imaging, its shape was examined by transmission electron microscopy (TEM). Moreover, the storage stability was analyzed based on ORN's entrapment efficiency during its storage time at room temperature (25°C) or in the freezer (-20°C). The *in vitro* study was assessed using ORN-based PDT on a human leukemia cell line (HL-60). Briefly, cells were cultured into a 96-well plate overnight and after 1 h incubation with 1 µM ORN were exposed to day-light LED (157 mW/cm2). The single-cell morphology was measured using super-resolution 3D Nanolive microscopy. Finally, the toxicity of this PDT procedure was studied with an *ex vivo* test and caspase-3 staining of ORN-treated ovarian tissue fragments.

Results: Fluorescence imaging (Fig. 2-a,b) shows the detectability of ORNs, and TEM morphology analysis (Fig. 2-c,d) confirmed the successful OR141-encapsulation into niosomes with the formation of an almost spherical shape. ORN shows no significant leakage at -20°C over 30-day storage, and there was about 90% leakage after one month for the ORN stored at 25°C (Fig. 2-e). Ovarian tissue fragments were employed to study *ex vivo* PDT toxicity (Fig. 3), the results approved that the procedure did not cause unwanted apoptosis on normal tissue (Fig. 4-a,b). On the other, *in vitro* PDT using 1 µM ORN showed complete eradication of HL-60 cancer cells (Fig. 4-c,d). Moreover, the morphological examination indicated that the cancer cell growth inhibition took place due to permanent membrane shrinking and cytoplasmic degradation after ORN-based PDT (Fig. 4-e,f).

Conclusion: Our approach demonstrated to be fluorescence detectable and acceptable storage stability in -20°C. ORN showed a considerable PDT effect on cancer cells, and this promising result shows the potential of ORN application for in situ and ex vivo PDT purging. Further investigations include the *ex vivo* PDT purging of contaminated fragments to determine the eradication rate of cancer cells. In addition, another niosomal PSs benefit is the ability to be targeted due to the surface functional groups and easy surface modifying with monoclonal antibodies, polymers, or ligands as targeting agents.

Keywords: Photodynamic Therapy, Vesicle, Drug delivery system, Photosensitizer, Cancer therapy

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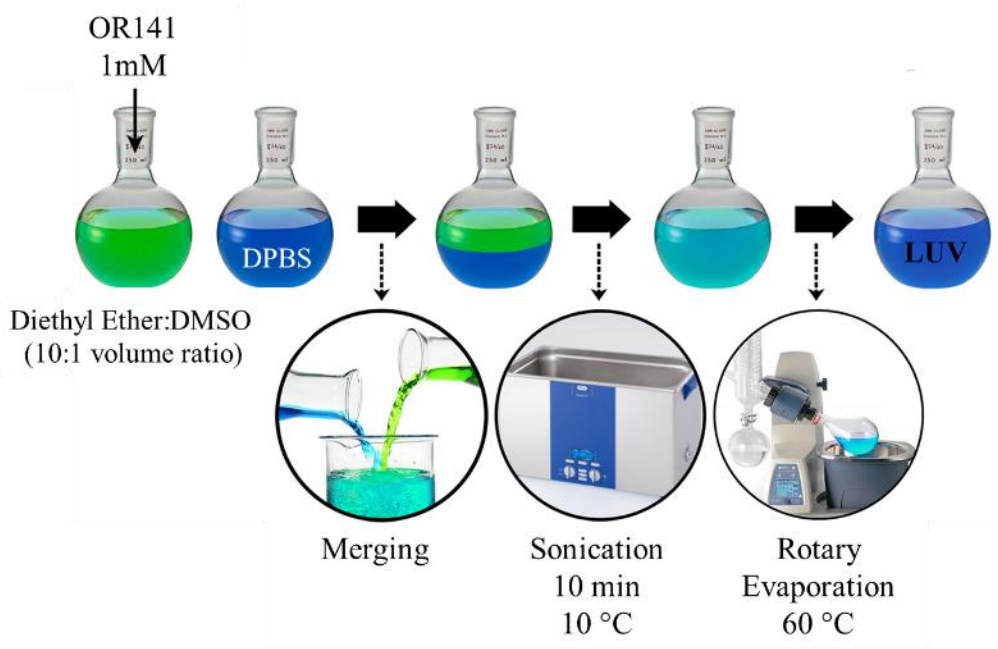

Fig. 1. Schematically illustration of the reversed-phase evaporation method for ORN preparation

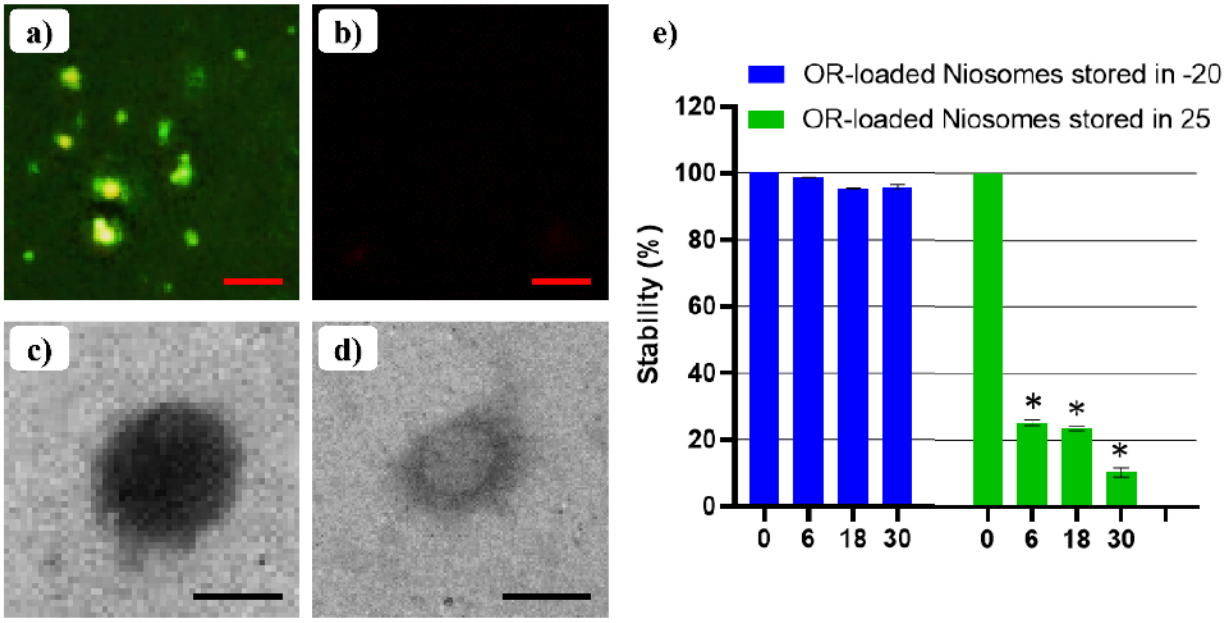

Fig. 2. Fluorescence imaging of ORN (a) and BLK (b) (Scale bar: 2 µm); TEM images of ORN (c) and BLK (d) (Scale bar: 100 nm); and the storage stability under different conditions (* p>0.0001)

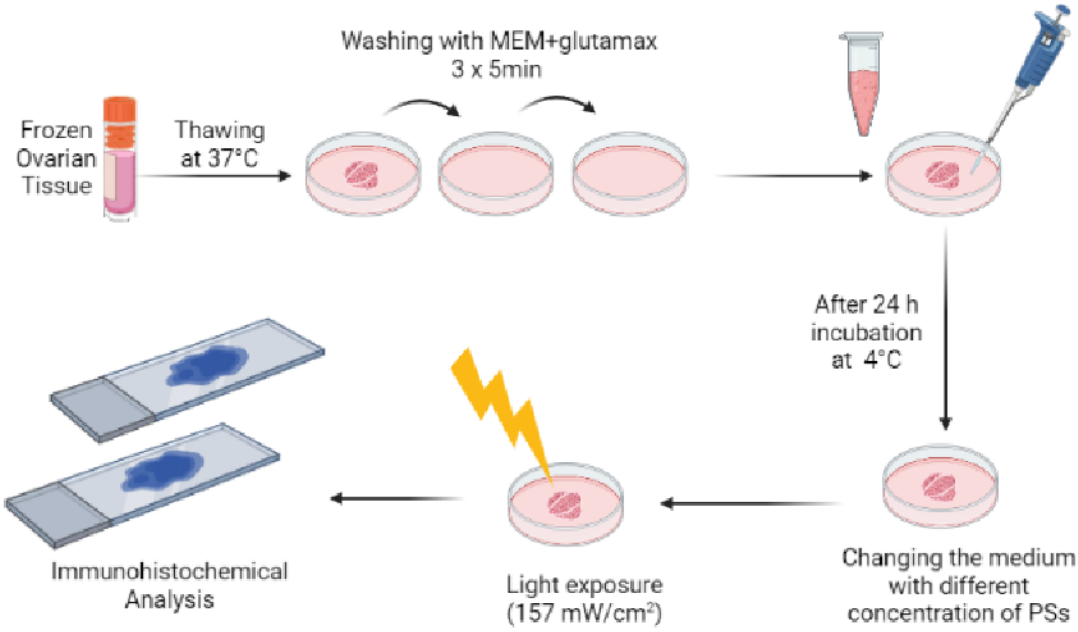

Fig.3. Schematically illustration of *ex vivo* PDT toxicity experiment

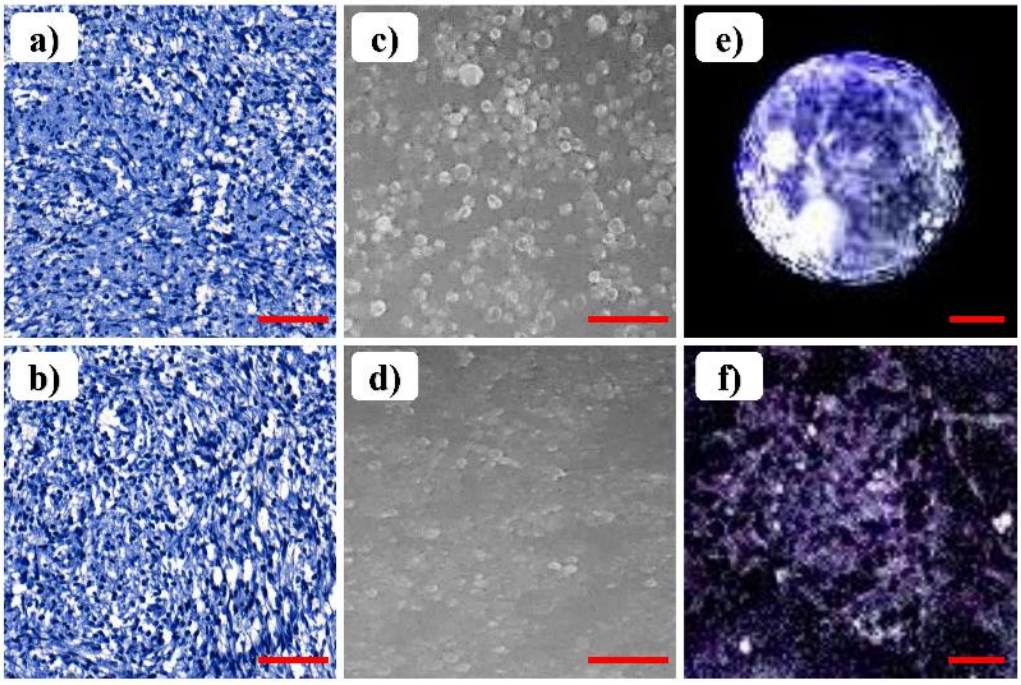

Fig. 4. Ex vivo toxicity test for ovarian tissues: untreated (a) and treated by 1µM ORN (b) (Scale bar: 50 µm); In vitro test on HL-60 cancer cell line: untreated (c) and treated by 1µM ORN (d) (Scale bar: 50 µm); and the morphological analysis of HL-60 cells: untreated (e) and treated by 1µM ORN (f) (Scale bar: 5 µm)