Supplementary information for Crosslinked Polymeric self-assemblies as an efficient strategy for photodynamic therapy on 3D cell culture

Ugo Till, Laure Gibot, Patricia Vicendo, Marie-Pierre Rols, Mireille Gaucher, Frédéric Violleau and Anne-Françoise Mingotaud

Content

| - | Fig. S1: ¹ H NMR spectrum of acrylate functionalized PEO-PCL | 2 |
|---|--|----|
| - | Fig.S2 : ¹ H NMR spectrum of crosslinked PEO-PCL micelles | 3 |
| - | Fig. S3: DLS analyses for PEO-PCL 5000-4000, PEO-PCL 2000-2800 | 4 |
| | and PEO-PCL 2000-7000 | |
| - | Fig. S4: Typical TEM photographs of polymer self-assemblies | 7 |
| | and analyses of the given images | |
| - | Fig. S5 : TEM follow-up of PEO-PCL 2000-7000 self-assemblies | 9 |
| | during fabrication process | |
| - | Fig S6: Typical DLS of self-assemblies before and after Pheo loading | 10 |
| - | Fig. S7: TEM images of Pheo-loaded PEO-PCL 2000-2800 micelles | 13 |
| - | Fig. S8 : Absorbance spectra of Pheo in different environments | 14 |
| - | Fig. S9: DLS analyses of stability vs time | 15 |
| - | Fig. S10 : Examples of DLS characterizations for addition | 19 |
| | of high ratios of THF | |
| - | Fig. S11: Influence of THF on PEO-PCL 2000-7000 self-assemblies | 20 |
| - | Fig. S12: Cytotoxicity and photocytotoxicity of Pheo encapsulated in | |
| | PEO-PCL 5000-4000 and 2000-7000 self-assemblies on normal human fibroblasts | 21 |
| - | Fig S13: validation of optically-based microscopic measurements | 21 |
| - | Fig. S14: metabolic ATP-based test performed on spheroids at D3 | 22 |
| | after the 3 irradiation process described in the manuscript | |
| - | Fig. S15: example of analysis of microscopy images of spheroids and controls | 23 |
| - | Fig. S16: Effect of unencapsulated Pheo on spheroids ([Pheo] $_0$ = 3.3 μ M) | 24 |
| - | Fig. S17: PDT efficiency at day 4 of the different self-assemblies on HCT 116 and FaDu cell lines | 25 |





Fig. S2: ¹H NMR spectrum of crosslinked PEO-PCL micelle after freeze-drying and re-suspension in CDCI3

Fig. S3: DLS analyses for freshly formed PEO-PCL 5000-4000, PEO-PCL 2000-2800 and PEO-PCL 2000-7000



For all DLS analyses, the upper graph corresponds to the correlograms, the middle one to the intensity mean analysis, and the lower one to the number mean analysis





Fig. S4: Typical TEM photographs of polymer self-assemblies and analyses of the given images

PEO-PCL 5000-4000 acrylate functionalized



PEO-PCL 2000-7000 acrylate functionalized



no analysis was performed, owing to the irregular shape of

the objects and their possible folding

PEO-PCL 2000-7000, crosslinked



no analysis was performed, owing to the irregular shape of

the objects and their possible folding













Fig. S5 : TEM follow-up of PEO-PCL 2000-7000 self-assemblies during fabrication process

| TEM | Water swelling | Sonication |
|---------------|---------------------|--|
| PEO-PCL 2000- | のなる理論では、 | 日本ながみなける |
| 7000 | 2012003126 | 的过去的方法 |
| | 84994-34954340 | Para Verson |
| | METRICAL | MUL DECT |
| | なりおかってするか | 1905 Ralles |
| | | 12 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 |
| | | JOY HALLON |
| | 200-am- | 200 mm |
| Analysis | Objects from 40 to | Objects from 20 to |
| | 400nm long 20-25 nm | 300nm long 20-30 nm |
| | | |
| | wide | wide |

Fig S6: Typical DLS of self-assemblies before and after Pheo loading For all DLS analyses, the upper graph corresponds to the correlograms, the middle one to the intensity mean analysis, and the lower one to the number mean analysis









Fig. S7: TEM images of Pheo-loaded unreacted PEO-PCL 2000-2800 micelles (A) and Pheo-loaded crosslinked PEO-PCL 2000-2800 micelles



Fig. S8 : Absorbance spectra of Pheo in different environments. o Free Pheo in water; --- Pheo in PEO-PCL 2000-7000; \Diamond Pheo in PEO-PCL 5000-4000 micelle; x Pheo in PEO-PCL 5000-4000 crosslinked micelle

Fig. S9: DLS analyses of stability vs time, comparison between DLS of freshly formed and 3-month old systems

For all DLS analyses, the upper graph corresponds to the correlograms, the middle one to the intensity mean analysis, and the lower one to the number mean analysis



Crosslinked system

uncrosslinked system (15 days)





Crosslinked PEO-PCL 5000-4000





Figure S10: Examples of DLS characterizations for addition of high ratios of THF



Fig. S11: Influence of THF on PEO-PCL 2000-7000 self-assemblies. o unreacted self-assemblies; ■ crosslinked systems. A intensity relative size; B number relative size; C scattered light intensity

On the intensity relative graph, the crosslinked system seems to remain stable at higher THF contents. The evolution for the unreacted system presents a decrease in size, whereas the swelling of the assembly should lead to the reverse. This behavior might be a sign of morphology evolution. The number relative analysis however shows an abrupt evolution of the crosslinked self-assembly at 30% THF. This might also be a sign of morphology evolution. As for the scattered light intensity, the behaviors for both systems are different for THF content up to 30%. The unreacted system exhibited a very strong increase of the intensity. This might be explained by a swelling of the assemblies. Indeed, a swelling of a worm-like system would lead to a main increase in its diameter and not necessary its length, leading to an increase in scattered light intensity but not in the average size since in this case the suggested size is obtained assuming the object is spherical.

Fig. S12: Cytotoxicity (A) and photocytotoxicity of Pheo encapsulated in PEO-PCL 5000-4000 (B) and 2000-7000 (C) self-assemblies on normal human fibroblasts



Fig. S13: validation of optically-based microscopic measurements Pearson test correlation:



Fig S14: metabolic ATP-based test performed on spheroids at D3 after the 3 irradiation process described in the manuscript



"Control" describes a spheroid grown in absence of polymer or Pheo, which is submitted to the same irradiation patterns than the other samples

Fig. S15: example of analysis of microscopy images of spheroids and controls. In the following figures, the pictures "control with or without irradiation" describes spheroids alone, without any polymer nor Pheophorbide

FaDu spheroids



200 mm

200 µur

| FaDu spheroids | Polymers alone with irradiation | Control with irradiation | Pheo alone with irradiation |
|-------------------|------------------------------------|-----------------------------|--------------------------------|
| PEO-PCL 2000-2800 | 200 µm | 200 µm | 200 µm |
| PEO-PCL 5000-4000 | 200 µm | Control without irradiation | Pheo alone without irradiation |
| PEO-PCI 2000-7000 | 200 µm | 200 µm | 200 µm |



Fig. S16: Effect of unencapsulated Pheo on spheroids ([Pheo]₀ = 3.3μ M)." Control" describes spheroids alone, without any polymer nor Pheophorbide, which is submitted to the same irradiation patterns than the other samples





Fig. S17: PDT efficiency at day 4 of the different self-assemblies on HCT 116 and FaDu cell lines