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# SUPPORTING INFORMATION

# Injectable peptide hydrogels for controlled-release of opioids

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## 1. Peptide synthesis

| Code | Sequences | Formula | HPLC tr (min) | $[M+H]^+$ | Yield (%) |
|---|---|---|---|---|---|
| **1** (MBG-1) | H-Phe-Glu-Phe-Gln-Phe-Lys-OH | $C_{43}H_{56}N_8O_{10}$ | 10.9 | 845.4202 | - |
| **2** | H-Phe-Glu-Phe-Gln-Phe-Lys-$NH_2$ | $C_{43}H_{57}N_9O_9$ | 11.6 | 844.4355 | 68 |
| **3** | Ac-Phe-Glu-Phe-Gln-Phe-Lys-$NH_2$ | $C_{45}H_{60}N_9O_{10}$ | 10.7 | 886.4322 | 59 |
| **4** | H-phe-glu-phe-gln-phe-lys-$NH_2$ | $C_{43}H_{57}N_9O_9$ | 11.2 | 844.4355 | 61 |
| **5** | H-Ile-Glu-Ile-Gln-Ile-Lys-$NH_2$ | $C_{34}H_{63}N_9O_9$ | 9.8 | 742.4822 | 62 |
| **6** | H-Leu-Glu-Leu-Gln-Leu-Lys-$NH_2$ | $C_{34}H_{63}N_9O_9$ | 10.1 | 742.4822 | 48 |
| **7** | H-Val-Glu-Val-Gln-Val-Lys-$NH_2$ | $C_{31}H_{57}N_9O_9$ | 8.3 | 700.4352 | 71 |
| **8** | H-Ala-Glu-Ala-Gln-Ala-Lys-$NH_2$ | $C_{25}H_{45}N_9O_9$ | 5.4 | 616.3413 | 54 |
| **9** | H-Cha-Glu-Cha-Gln-Cha-Lys-$NH_2$ | $C_{43}H_{75}N_9O_9$ | 12.8 | 862.5760 | 28 |

## 2. Dynamic rheometry profiles of hydrogelators 2, 4, 5 and 9

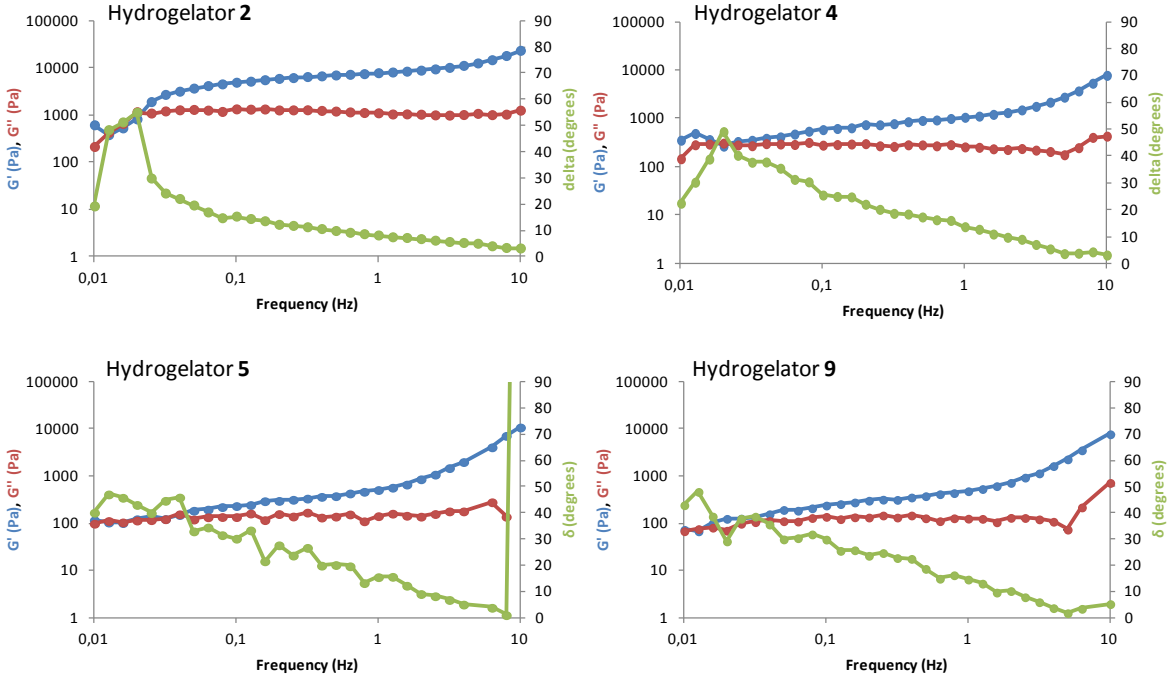



***Figure S1***. Dynamic frequency sweep data for the hydrogelators **2, 4, 5** and **9** at a concentration of 2% w/v in PBS.

## 3. Transmission electron microscopy images

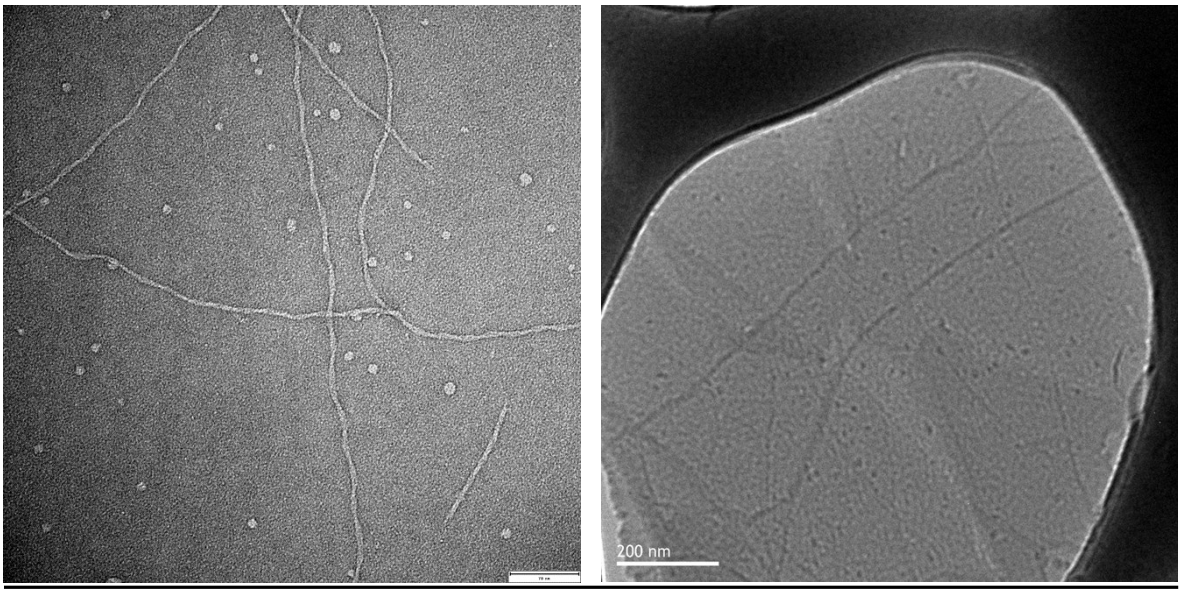



***Figure S2.*** TEM image with negative staining of hydrogelator **2** (left, scale bar 70 nm) and cryogenic TEM picture for hydrogel **4** (right, scale bar 200 nm).

## 4. *In vitro* stability

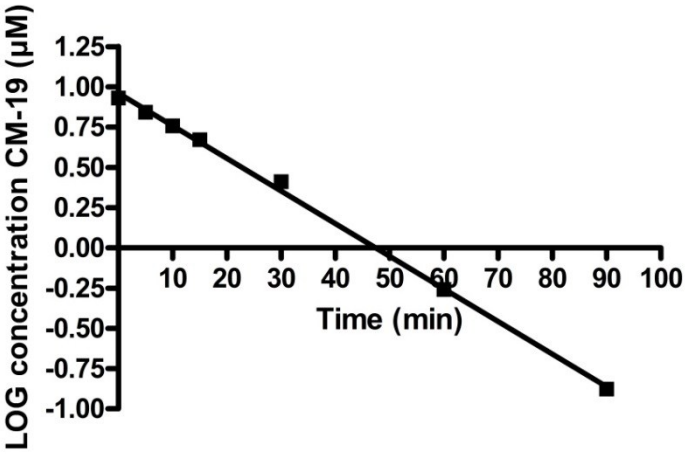
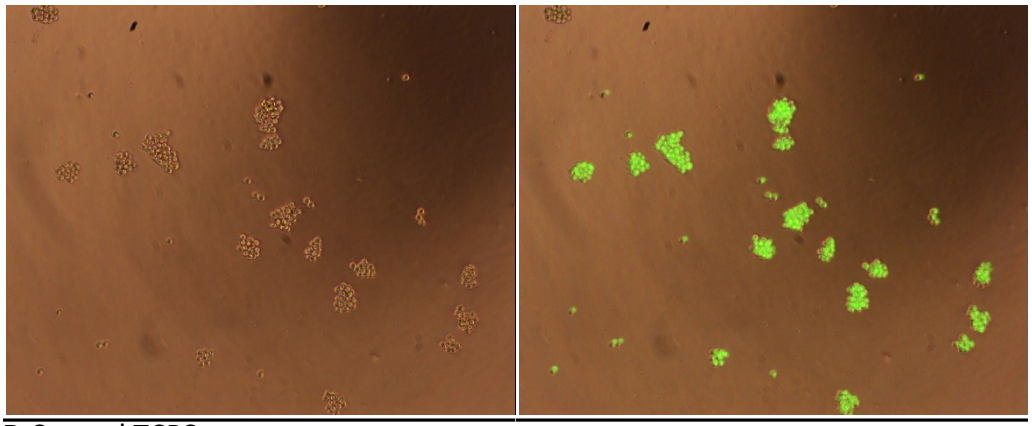


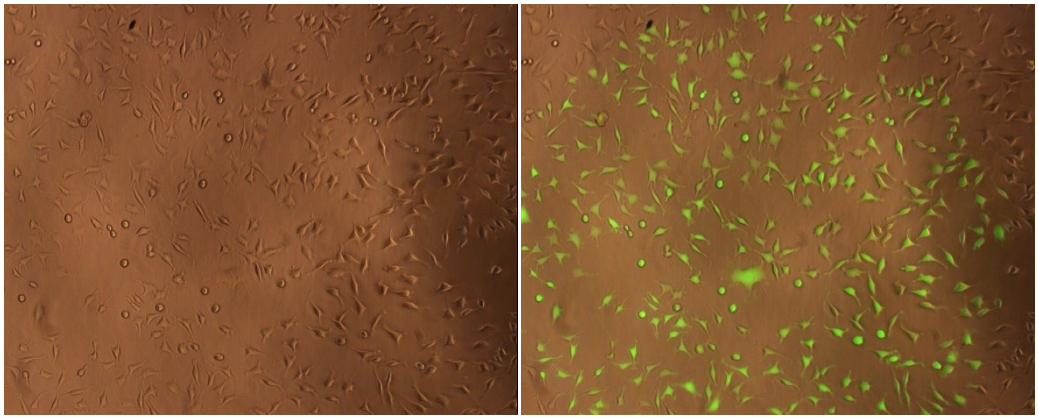

***Figure S3.*** Plasma concentration of hydrogelator **2** as a function of time (semi Log). Samples were taken after 0, 5, 10, 15, 30, 60 and 90 minutes. The experiment was performed on three independent samples. The average result is plotted allowing to determine the equation which describe the degradation as a function of time as y= -0,02024x + 0,9611 given an **half-life** of **14.71 ± 0.15 min**.

## 5. Cytotoxicity

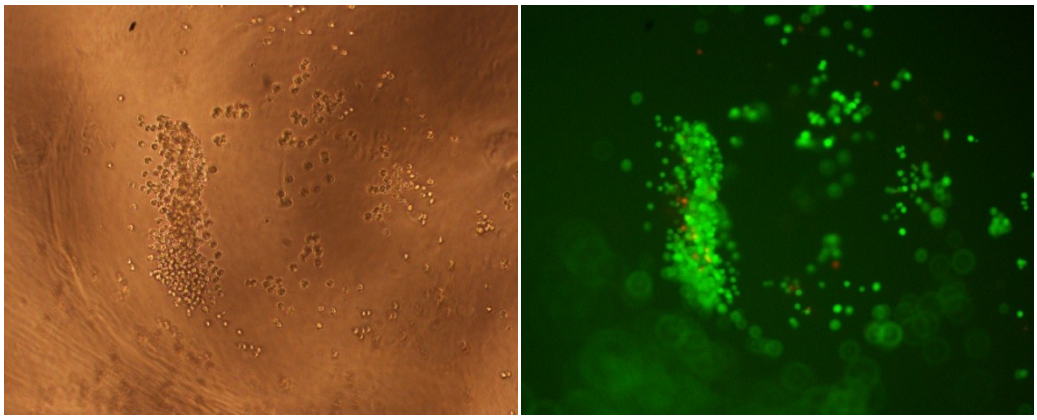
**A**: ULA



**B**:Control TCPS



**C**: hydrogelator **2**



**D**: hydrogelator **4**

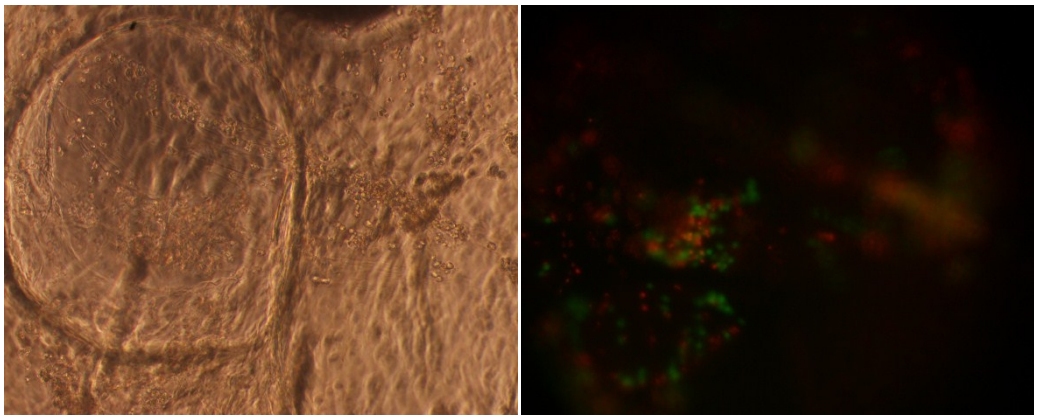


***Figure S4.*** Qualitative cytotoxicity determined using a two-color fluorescence cell viability assay that is based on the simultaneous determination of live and dead cells. In the left, Scale bar calculation: 200μm is represented as 16.3% of the width of the original image.

The LIVE/DEAD® Viability/Cytotoxicity Assay Kit provides a two-color fluorescence cell viability assay that is based on the simultaneous determination of live and dead cells. Live cells have intracellular esterases that convert nonfluorescent, cell-permeable calcein acetoxymethyl (calcein AM) to the intensly fluorescent calcein. The polyanionic dye calcein is well retained within live cells, producing intense uniform green fluorescence in live cells, whereas the ethidium homodimer-1 (EthD-1) enters damaged cells and undergoes strongly enhanced fluorescence upon binding to nucleic acids thereby producing a bright red fluorescence in damaged or dead cells. Cells were plated in duplicate (1 x $10^5$ cell/well in 16 well plates) on control TCPS culture plate, control ULA plate and ULA plate containing the nanostructured hydrogels (including hydrogels **2** and **4**). Plates were incubated in 5% $CO_2$ at 37°C for 1 day to allow cell attachment and spreading to occur. Media was removed and each well was washed with Gibco® solution to remove serum proteins. A stock solution of (1 mM) calcein AM and (2 mM) ethidium homodimer in PBS was prepared according to the live/dead assay (Molecular Probes L3224) package instructions, and 100 μL of this stock was added to each well. Photographs of cells were obtained using 10 times magnification on Nikon microscope.

## 6. Injectable peptide hydrogels as a controlled drug delivery system: movie

Amphipathic α-peptide low molecular weight gelators have been developed as new controlled drug delivery systems. Prior to use *in vivo* experiments, injectability of the hydrogels was tested and immediate re-gelation was confirmed after injection through a 25G needle destined for subcutaneous injection. The injection and re-gelation steps could be repeated several times using the same hydrogel sample.

http://youtu.be/zXTQWdMY5CM

## 7. Dynamic rheometry profiles of morphine co-formulated with hydrogelator 2

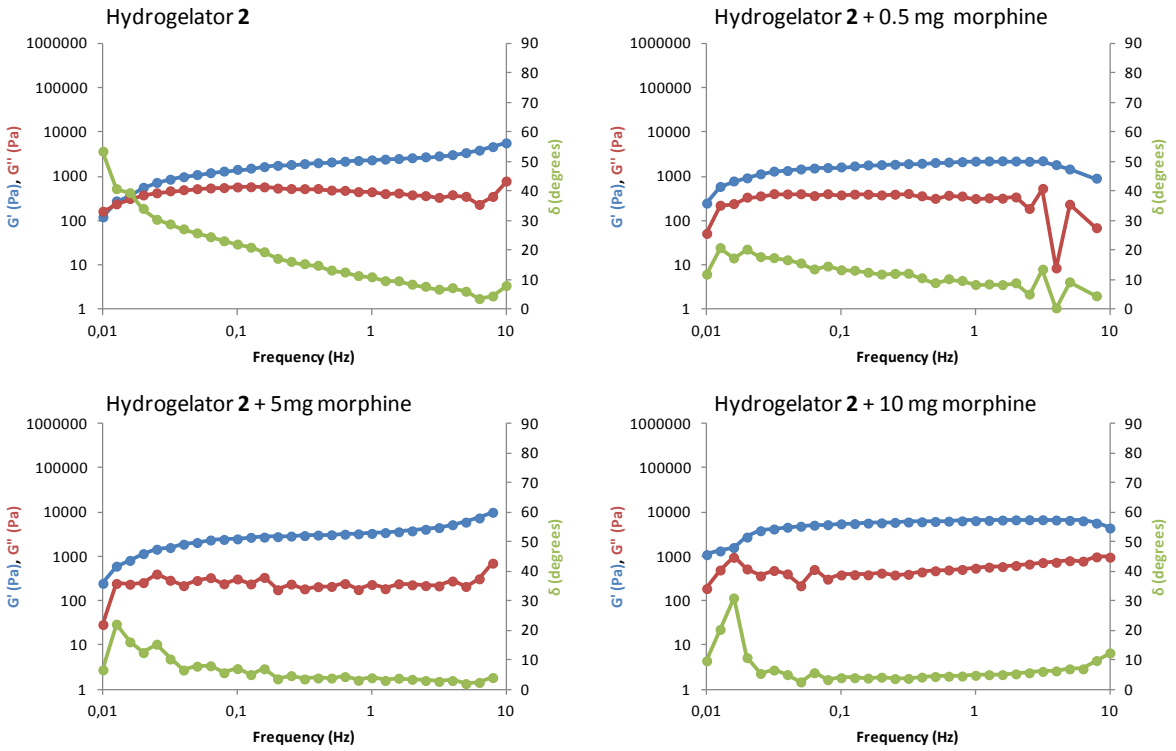



***Figure S5***. Dynamic frequency sweep data for the hydrogelator **2** (2% w/v) and hydrogelator **2** (2% w/v) co-formulated with 0.5 (10.3mM), 5 (103mM) and 10 mg (206 mM) of morphine in physiological saline.