Electronic Supplementary Information

Preparation and characterization of nanofunctionalized alginate/methacrylated gelatin hybrid hydrogels

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1. Experimental

1.1. Material

Alginic acid sodium salt (SA) from brown algae (M/G ≈ 1.56) was purchased from Sigma Aldrich. The average viscosity molecular weight of the polymer in pure water M_v was 1.69 10^5 g.mol^{-1} was calculated at 20°C in 0.1 M NaCl using the Mark–Houwink–Sakurada correlation: $[\eta] = KM_v^{\alpha}$ Where α = 0.92 and k = 7.3 10^{-5} . [1]

Calcium chloride dihydrate was obtained from VWR (International, Leuven, Belgium).

Gelatin (type A, 300 bloom from porcine skin), methacrylic anhydride (MA), photoinitiator (PI) 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone and phosphate buffered saline tablets (PBS) were purchased from Sigma-Aldrich (Chemie, Steinheim, Germany). Rapeseed lecithin were acquired from Solae Europe SA society (Geneva-Switzerland).

1.2. Nanoliposomes preparation

Rapeseed lecithin were dissolved in distilled water with a concentration of 5% (w/v). The suspension was mixed for 4 h under agitation at inert atmosphere (nitrogen) and then sonicated at 40 kHz and 40% of full power for 300 s (1 s on / 1 s off) to get a homogeneous solution. Finally, the liposomal suspension was stored in the dark at 4°C until use. In order to functionalize the studied systems, 43 µl of nanoliposomes were added to each 5mL of the polymer solutions. The size distribution (Mean diameter and Polydispersity Index) and electrophoretic mobility (µE) of the liposome dispersions were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, UK). Prior to measuring size, the samples were diluted (1:400) into a distilled water. Measurements were made at 37 °C with a fixed angle of 173°. Three readings were made per sample and each measurement was repeated three times.

The average particle size of nanoliposomes was 110 nm with an electrophoretic mobility of -3.41mV (Fig. S1).

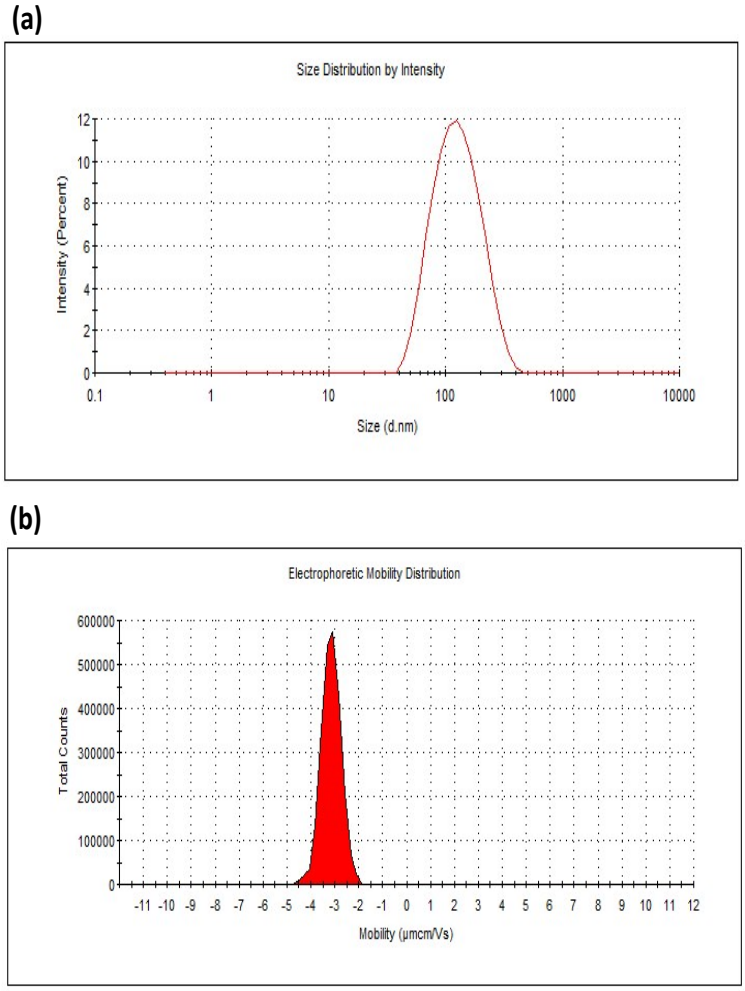

Fig.S1. Size distribution by intensity of the prepared nanoliposomes (a) and electrophoretic mobility distribution (b).

1.3. Synthesis of GelMA

Gelatin was dispersed at 10% (w/v) in PBS and stirred at 60 °C until fully dissolved. Then, 8 mL of MA was added very slowly and dropwisly under stirring. After 3h, the reaction was stopped following a dilution 5X using warm phosphate buffered saline. Diluted GelMA was then dialyzed at 40-50 °C for one week against distilled water using a dialysis membrane (Spectro/Por molecular porous membrane tubing, MWCO 12-14,000, SpectrumLabs, Inc., Rancho Dominguez, CA, USA). The solution was then lyophilized during 1 week. GelMA was developed with high methacrylation degree (~70%).[2]

1.4. Solutions preparation

To prepare alginate solution, 6 g of alginic acid sodium salt was dispersed into stirred 100 mL double distilled water. After complete solubilization, the alginate solution was degassed to remove air bubbles before its use.

GelMA solution (30 %, m/v) was prepared by dissolving the freeze-dried powder into a PBS solution at 40 °C. Then, 1% of PI was added and the temperature was increased to 80 °C to allow its solubilization.

The alginate/GelMA solution was prepared at 40 °C by mixing alginate with GelMA, the final concentrations is 2 % and 20% (m/v), respectively. Then 1 % (m/v) of PI was added to the final mixture.

1.5. Hydrogels preparation

1.5.1. Alginate hydrogel

Alginate hydrogel was synthetized by pouring 2mL of the solution slowly on 5 mL of calcium chloride solution (2% m/v) in petri dishes. The obtained hydrogel was incubated into the $CaCl_2$ for 24 h at 4°C in order to complete the reticulation process and then washed with distilled water before use.

1.5.2. GelMA Hydrogel

GelMA was crosslinked after poured on a specific mold with the controlled dimensions and then exposed to UV light (360-480 nm) for 240 s. The PI absorbs the UV light and transform the solution onto gel. The obtained hydrogel was then washed with distilled water before use.

1.5.3. Alginate/GelMA IPN hydrogel

The crosslinking process occurs in two stages. First, 2 mL of the mixture was poured on 5 mL of $CaCl_2$ solution (2%, m/v) in order to allow alginate cross-linking then the obtained semi-IPN was exposed to UV light for 240 s to allow the free radicals photopolymerization of the GelMA. The final hydrogel was then rinsed with PBS to remove the excess of $CaCl_2$.

1.6. Characterization of the solutions

1.6.1. Zeta potential measurements

Zeta potential of the various solutions were measured with Zetasizer Nano ZS (Malvern Instruments Ltd., UK) using dynamic light scattering (DLS). The determined potential is an important parameter to analyze the effect of the nanoparticles in suspension. The samples were diluted (1:2) and introduced into disposable capillary cells equipped with gold electrodes designed to afford maximum zeta potential measurement capability. All measurements were carried out at 37 °C with a fixed angle of 173°. The presented values are the mean of three measurements.

1.6.2. Surface tension

The surface tension of aqueous solutions of alginate, GelMA and the mixture of the two polymers, with and without liposomes was measured using a tensiometer Wilhelmy plate type (krüss GmbH, Hamburg, Germany) at a constant temperature of 37 °C. The platinum plate was cleaned with a flame treatment in order to remove any contaminating substances. The plate was then inserted into the solution placed in a circular glass vessel with an immersion depth of 2.0 mm. The surface tension was measured with a crosshead speed of 10 mm / min and a probe sensitivity of 0.005g and a duration of 60 seconds. The presented values of the surface tension are the mean of three measurements.

1.7. Characterization of the prepared hydrogels

1.7.1. Contact angle

The wettability of all samples is evaluated by the measurement of the contact angle formed on the surface of the hydrogel using the sessil drop method. Before starting the test, the gel is taken out of distilled water and dried superficially in an oven at body temperature of 37 °C. After reaching the target temperature, uniform distilled water drops of 0.75 µl were deposited on the gels using a microsyringe. The liquid on the surface of the hydrogel form a static angle measured with a goniometer (Digidrop, GBX instruments, France) equipped with an image analysis software (Windrop, France). All data presented the mean values of three replicates performed on the surface of each hydrogel.

1.7.2. Surface energy measurement

The surface energy of the hydrogels is calculated using the Owens-Wendt theory based on the contact angle measurements. It includes two constituants, the dispersion γ^d and the polar γ^p, composing the total of surface energy. [3,4]

$$\gamma^T = \gamma^d + \gamma^p \quad (1)$$

Owens and Wendt introduce the contact angle θ toward the equation and gives the following relation (eq.2):

$$\gamma_L (1 + cos\theta) = 2\sqrt{\gamma_S^d}\sqrt{\gamma_L^d} + \sqrt{\gamma_S^p}\sqrt{\gamma_L^p} \quad (2)$$

where γ_L is the liquid surface tension, γ_S^d and γ_S^p form respectively the dispersive and the polar components of the hydrogel, γ_L^d is the dispersive component of the liquid and γ_L^p is the polar component of the liquid. The total surface energy of each hydrogel is determined before and after the nanofunctionalization of hydrogels.

1.7.3. Hydrogels swelling analysis

To study swelling properties, hydrogels were frozen and lyophilized in order to obtain the hydrogels dry mass. The lyophilized hydrogels were then incubated in pure water for 24 and 48 hours at 37°C and their weights was measured at each time to determine the wet mass. The swelling ratio was calculated as the ratio of wet mass to dry mass. Measurements were repeated three times.

1.7.4. Conductivity

The conductivity measurements were performed using a conductimeter (HD 2156.1, Delta OHM). The hydrogel was removed from the distilled water and then dried. The sample was then placed on a contact probe and the conductivity was displayed on a LCD display.

1.7.5. Scanning Electron Microscopy

The surface morphology of the different hydrogels were characterized by Quanta 200 high resolution scanning electron microscope low vacuum mode (FEI-Japon). The use of "low vacuum" mode presents powerful tools for the observation of the surface topography of biological materials without sputter-coated. It also preserve the delicate samples from the electron beam damaging. The maximal resolution attained, employing an electron beam spot size of 7, could be lower than 5 nanometers. A large field detector (LFD) was used in order to execute this analysis. The squared shaped samples with dimensions 9mm x 9mm were inserted and maintained in a holder inside the SEM chamber and the tests were performed at laboratory temperature of 25°C with a relative humidity of 50%. A partial vacuum was created within the chamber and the air was evacuated using a pump which provide a regular pression of 60 mbar.

The images were taken from a distance of 10 mm at an accelerating voltage of 15 kV. The pictures were provided utilizing logical “xT microscope server”.

1.7.7. Drug release

To evaluate the potential use of the synthetized matrixes for drug delivery applications, the prepared hydrogels were incubated in 10 ml of water at 37 °C. At scheduled time intervals, 1 mL of the external media was pipetted after gentle stirring and the volume was replaced by 1 mL of fresh water to keep a constant volume. The released amount of curcumin was determined using the method developed by Hasan et al., 2014.[5]

Briefly, the concentration of the released curcumin was measured by a reverse-phase HPLC system (Shimadzu, Kyoto, Japan) equipped with a quaternary pump (LC-20AD), an auto-injector (SIL-20AC HT), a UV–vis photodiode array detector (UV–vis PDA, SPD-M20A), a Zorbex SB-C18 column (5 µm, 4.6 mm × 250 mm) and Labsolution data software. Suspension was analyzed in isocratic mode using methanol (v/v, 5%), acetic acid 2% (v/v, 30%) and acetonitrile (v/v, 65%) at a flow rate of 0.5 mL min^{-1}. The amount of aliquot injected onto an AlltimaTM [HP C18, 5 µm (250 mm × 4.6 mm i.d.) column (GRACE, Deerfield, IL, USA)] at 25◦C is 20 µL. Detection of curcumin was performed at 425 nm after 8 min and 49 s.

1.7.7. Viscoelastic measurements

Dynamic viscoelastic measurements were carried out using a kinexus pro rheometer (Malvern Instruments, Orsay, France) equipped with a plate-and-plate geometry (20 mm).

A dynamic frequency sweep test from 0.001 to 100 Hz was performed to determine the dynamic storage (G’) and viscous (G”) modulus, at a strain rate confirmed to be in the linear viscoelastic range for each type of hydrogel by a prior strain amplitude sweep. During the rheological experiments, the temperature was maintained at 37 °C and the measuring system

was covered with a humidity chamber to minimize water evaporation. Three different hydrogel disks were tested for each type of hydrogel with the same experimental settings; average values are presented.

1.8. Statistical analysis

Results were analyzed by analysis of variance (ANOVA) with 95 % significance level using SigmaPlot software (SigmaPlot Version 11.0, Systat Software Inc., San Jose, CA, USA).

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