Supplementary Information

BIOMIMETIC CELLULOSE-BASED SUPERABSORBENT HYDROGELS FOR TREATING OBESITY

Marta Madaghiele[1*], Christian Demitri[1], Ivo Surano[1], Alessandra Silvestri[2], Milena Vitale[2], Eliana Panteca[3,4], Yishai Zohar[3,4], Maria Rescigno[2,5], Alessandro Sannino[1*]

[1] Department of Engineering for Innovation, University of Salento
Via per Monteroni, 73100 Lecce, Italy

[2] IRCCS Humanitas Research Hospital
Via Manzoni 56, 20089 Rozzano, Milan, Italy

[3,4] Gelesis
Boston, MA, 02116 USA & Calimera, Lecce 73021, Italy

[5] Department of Biomedical Sciences, Humanitas University
Via Rita Levi Montalcini 4, 20072 Pieve Emanuele, Milan, Italy

[*]Corresponding authors:
Marta Madaghiele
Department of Engineering for Innovation, University of Salento
Via per Monteroni, Lecce 73100, Italy
Email: marta.madaghiele@unisalento.it

Alessandro Sannino
Department of Engineering for Innovation, University of Salento
Via per Monteroni, Lecce 73100, Italy
Email: alessandro.sannino@unisalento.it

1. Supplementary Methods

Analysis of CMC types

Size exclusion chromatography (SEC)

SEC was performed to assess the molecular weight distributions of CMC-L and CMC-H. A Waters e2695 liquid chromatograph coupled with Waters 2414 differential refractometer detector (RID) were used for the measurements. Separation was performed in two Agilent PL-aquagel-OH Mixed-H columns (300 x 7.5 mm, 8 µm particle size) in series, preceded by Agilent PL-aquagel-OH Guard column (50 x 7.5 mm, 8 µm particle size). A pH 7 aqueous buffer (0.2 M sodium nitrate and 0.01 M sodium phosphate monobasic dihydrate in purified water) was used as the mobile phase at a flow rate of 1 mL/min and 35°C. A calibration curve was obtained by analyzing a series of pullulan standards (Ready Cal-Kit Pullulan high, PSS Polymer Standards Service GmbH) in the molecular weight range from 6,100 to 1,220,000 g/mol, solubilized in purified water at a concentration of 0.15% w/w. CMC samples (n=4 for each type), dissolved in purified water overnight at a concentration of 0.15% w/w, were then analyzed. Data were collected and processed using the Empower3 software (Waters Corporation) to determine the weight average molecular weight (M_w), the number average molecular weight (M_n) and the polydispersity index ($PDI=M_w/M_n$), by means of the integration algorithm ApexTrack.

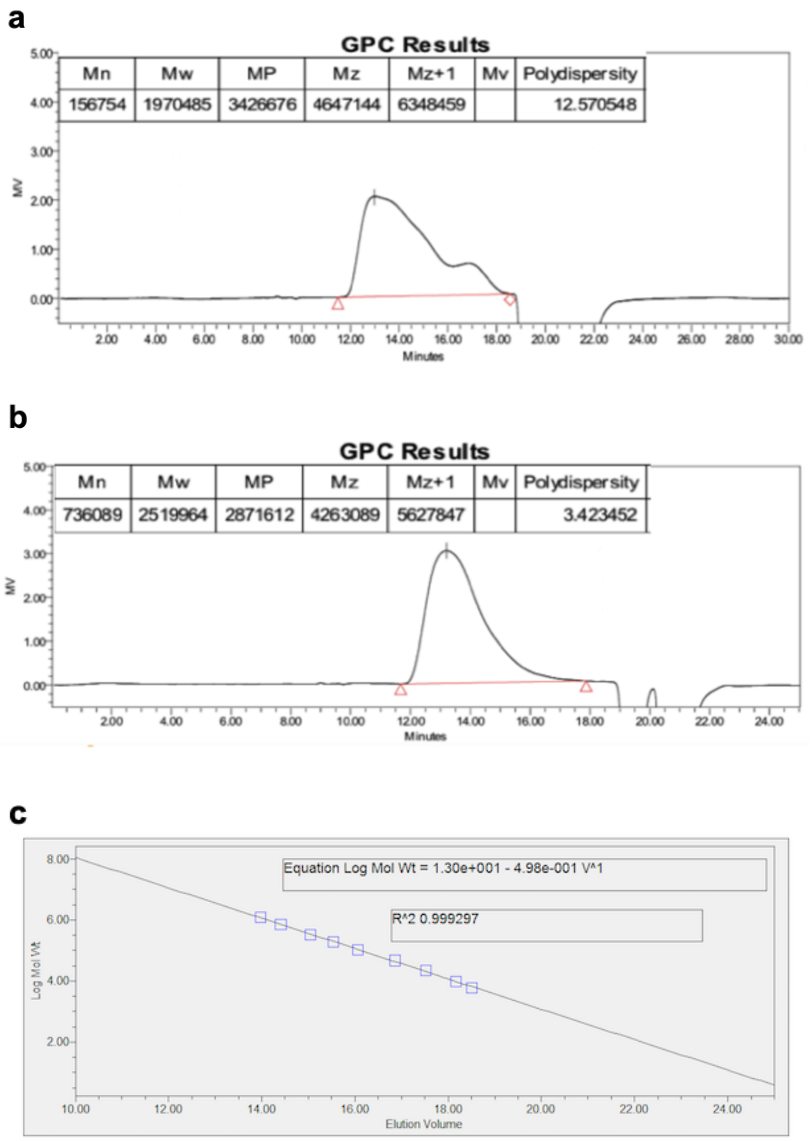
Rheological measurements (hysteresis loop tests)

For each CMC type, rheological measurements on CMC solutions (2% w/v) were performed at 25°C, using a Discovery HR-1 rotational rheometer (TA Instruments) with the cone-and-plate geometry (diameter 40 mm, gap 0.026 mm). In order to evaluate the thixotropic behavior of the CMC solutions, a hysteresis loop test was carried out, which consisted of the following loading/unloading program, under controlled shear rate mode: (a) increase of the shear rate from 0.1 to 100 s^{-1} in 5 minutes; (b) constant shear rate of 100 s^{-1} for 1 minute; (c) decrease of the shear rate from 100 to 0.1 s^{-1} in 5 minutes. Considering the plot of shear stress *vs.* shear rate, the upward (loading) and the downward (unloading) curves do not superimpose, with the downward curve being located below the upward one. A dimensionless thixotropic index (TI) was thus calculated as:

$$TI = \frac{A_{up} - A_{down}}{A_{up}} \times 100 \qquad \text{(Eq. S1)}$$

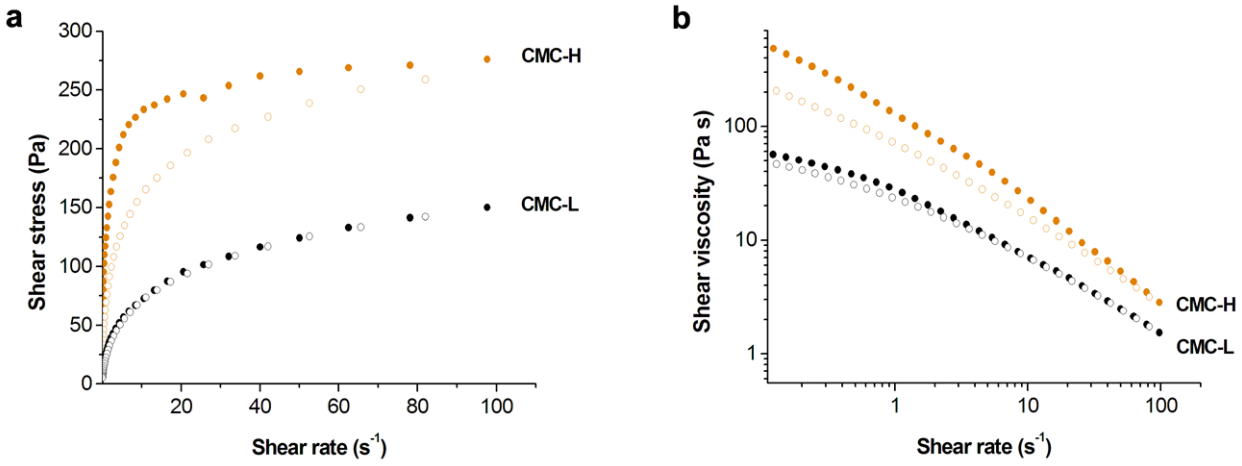
where A_{up} and A_{down} are the areas under the upward and the downward curves, respectively, and (A_{up} - A_{down}) represents the hysteresis area.

2. Supplementary Figures (Supplementary Figs. 1-3)



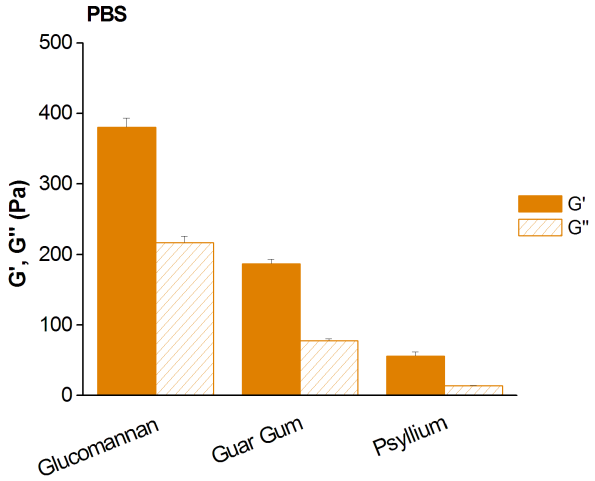
Supplementary Fig. 1. Results of size exclusion chromatography.

Typical chromatograms of CMC-L **(a)** and CMC-H **(b)**, indicating a larger M_w and a narrower molecular weight distribution of CMC-H compared to CMC-L. **(c)** Calibration curve obtained from pullulan standards and used for the analysis of the CMC samples.



Supplementary Fig. 2. Results of hysteresis loop tests performed on aqueous CMC solutions (2% w/v).

Typical plots of shear stress **(a)** and shear viscosity **(b)** of CMC-L and CMC-H solutions, as a function of the shear rate: full symbols indicate the upward/loading curve, while empty symbols indicate the downward/unloading curve. The hysteresis area in **(a)** gives an idea of the extent of thixotropy, which is calculated according to Eq. S1.



Supplementary Fig. 3. Elastic modulus G' and viscous modulus G'' (at 10 rad/s) of functional fibers upon solubilization in phosphate buffered saline (PBS) at 37°C (2% w/v).

Results are the mean ± SD of three independent measurements. All detected differences in elasticity (G') among glucomannan, guar gum and psyllium were significant (p<0.0001).

3. Supplementary Tables 1-2

Supplementary Table 1. Simulated Gastric Fluid (SGF), Simulated Intestinal Fluid (SIF) and Simulated Colonic Fluid (SCF) used in this study.

* Used enzymes were pepsin (1:3000), pancreatin from porcine pancreas (8xUSP) and pectinase from *Aspergillus niger* (>1 U/mg).

	SGF	SIF	SCF
COMPOSITION			
Sodium chloride (g)	2.0	-	-
Hydrochloric acid 1 M (mL)	84.0	-	-
Purified water (mL)	-	750.0	750.0
Monobasic potassium phosphate (g)	-	6.8	6.8
Sodium hydroxide 0.2 N (g)	-	117.0	117.0
Pepsin* (g)	3.2	-	-
Pancreatin* (g)	-	10.0	-
Pectinase* (g)	-	-	10.0
Adjust pH and/or volume			
Sodium hydroxide 0.2 N to (or hydrochloric acid 0.2N)	-	pH 6.8	pH 6.8
Purified water to (L)	1.0	1.0	1.0
PROPERTIES			
pH	1.1	6.8	6.8
Ionic strength (mM)	118	72	72

Supplementary Table 2. Simulated GI model to assess absorption (MUR) and elasticity (G') variations of the CB-SAHs at 37°C.

* Each time point represents an individual sample (e.g. the sample tested at 120 min is previously immersed in SGF/water 1/8 up to 60 minutes and then immersed in SGF/water 1/4 for additional 60 minutes, before MUR and G' assessment).

Simulated GI environment	Simulated GI interval (min)	Simulating GI medium	Testing time (min)*
Stomach	0-60	SGF/water 1/8	5
			15
			30
			60
	60-120	SGF/water 1/4	120
	120-180	SGF	180
Small Intestine	180-300	SIF	240
			300
Large Intestine	300-360	SCF	360

4. Supplementary Video

Supplementary Video 1. Time-lapse video of the hydration of a single CB-SAH granule (GelA in SGF/water 1/8 v/v) over 10 minutes.