



# Thermo-responsive properties of methylcellulose hydrogels for cell sheet engineering



Nicola Contessi<sup>a,b</sup>, Lina Altomare<sup>a,b</sup>, Andrea Filippini<sup>a</sup>, Silvia Farè<sup>a,b,\*</sup>

<sup>a</sup> Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy

<sup>b</sup> National Interuniversity Consortium of Materials Science and Technology (INSTM), Florence, Italy

## ARTICLE INFO

### Article history:

Received 3 March 2017

Received in revised form 11 May 2017

Accepted 2 July 2017

Available online 17 July 2017

### Keywords:

Smart materials

Methylcellulose

Rheology

UV spectroscopy

Cell sheet engineering

## ABSTRACT

Methylcellulose (MC) hydrogels change their affinity to water depending on their temperature and can thus be used as substrates for cell sheet engineering. In this work, we characterize the thermo-responsive properties of 8% w/v MC hydrogels, produced in two saline solutions (*i.e.*, Na<sub>2</sub>SO<sub>4</sub> and phosphate buffered saline) at different concentrations, by investigating the rheological properties and the UV-absorbance in function of temperature. Both rheological and UV-spectroscopy tests showed that the addition of salts to MC hydrogels allowed lowering the LCST of the MC hydrogel; moreover, hydrogels produced in 0.1 M Na<sub>2</sub>SO<sub>4</sub> or PBS 20 g/L were proved to be particularly promising for cell sheet engineering application, showing a LCST below 37 °C.

© 2017 Elsevier B.V. All rights reserved.

## 1. Introduction

Smart hydrogels reversibly change their properties when exposed to an external driven force as pH or temperature variation [1]. Methylcellulose (MC) is a polysaccharide derived from cellulose by partial substitution of hydrophilic hydroxyl groups with hydrophobic methoxy groups. MC dissolved in aqueous solvents forms reverse thermo-responsive smart hydrogels that undergo a sol-gel transition when heated, associated with change from hydrophilicity at low temperatures (hydrogen bonds between water and MC hydroxyl groups) to hydrophobicity at higher temperatures (loss of hydration due to methoxy groups exposition and higher interaction among them) [2]. This sol-gel transition is characterised by a Low Critical Solution Temperature (LCST), tuneable by varying MC degree of substitution, concentration [3], and by adding salts in the solution (*i.e.*, salting-in or salting-out) [4]. These features, together with ease of availability, low-cost and biocompatibility, make MC a promising material for regenerative medicine [5] and cell sheet engineering [6]. In the latter case, cells cultured *in vitro* adhere and proliferate on MC at 37 °C (*i.e.*, hydrophobic substrate) while they spontaneously detach at lower temperature (*i.e.*, hydrophilic substrate) preserving the inter-

cellular connections and ECM, thus forming cell sheets to be directly applied on pathological tissues without implanted supporting materials (*i.e.*, scaffold-free tissue engineering). Typical LCST of 1% w/v MC hydrogels in distilled water is >50 °C [7], incompatible with cell culture conditions; thus, tuning the LCST by either increasing MC concentration and/or adding salts to the solvent is a key aspect when producing a substrate for cell sheet engineering.

Here, we propose relatively highly concentrated MC hydrogels (*i.e.*, 8% w/v), produced in differently concentrated ions solutions and we investigate their LCST by rheological tests, proposing an innovative investigation of LCTS for hydrogels with  $G' > G''$ , and UV spectroscopy. Moreover, LCST values obtained by the two techniques were compared to verify the possibility to use only one of them for LCST investigation on MC hydrogels.

## 2. Materials and methods

### 2.1. Synthesis

METHOCEL powder (A4M, Dow Chemical Company) 8% w/v was dissolved at 55 °C in different distilled water saline solutions (Table 1): phosphate buffered saline (PBS, Sigma Aldrich) at 10 g/L (MC\_PBS10) and 20 g/L (MC\_PBS20), 0.05 M (MC\_Na005) and 0.1 M (MC\_Na01) sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>, Sigma Aldrich) or distilled water as control (MC\_water). After complete powder dissolution, solutions were sealed in petri dishes (Ø = 35 mm) and stored at 4 °C for 24 h to allow MC complete hydration [8].

\* Corresponding author at: Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Piazza Leonardo da Vinci, 32-20133 Milan (IT), Italy.

E-mail address: [silvia.fare@polimi.it](mailto:silvia.fare@polimi.it) (S. Farè).

**Table 1**Methylcellulose hydrogels and their characteristic gelation temperatures (LCST) measured by rheology ( $G'$ ,  $\eta^*$ ) and UV spectroscopy.

Sample	MC concentration [%w/v]	Salt concentration	LCST/rheology $G'$ [°C]	LCST/rheology $\eta^*$ [°C]	LCST/UV [°C]
MC_water	8	–	38.7 ± 0.5	39.1 ± 0.4	42.5 ± 0.3
MC_PBS10	8	PBS 10 g/L	39.9 ± 0.8	40.1 ± 0.7	39.4 ± 0.3
MC_PBS20	8	PBS 20 g/L	37.9 ± 0.3	38.1 ± 0.4	35.4 ± 0.3
MC_Na005	8	Na <sub>2</sub> SO <sub>4</sub> 0.05 M	36.9 ± 0.3	37.2 ± 0.5	33.4 ± 0.2
MC_Na01	8	Na <sub>2</sub> SO <sub>4</sub> 0.1 M	37.2 ± 0.5	37.6 ± 0.6	31.9 ± 0.3

## 2.2. Weight variation

Weight variation tests were performed in distilled water at 37 °C. Hydrogels ( $n = 3$ ) after 24 h of hydration were weighted ( $w_0$ ) and immersed in distilled water at 37 °C; at established time points, up to 7 days, hydrogels were weighted ( $w_t$ ) and the percentage weight variation ( $\Delta W\%$ ) was calculated as (1)

$$\Delta W\% = \frac{W_t - W_0}{W_0} \cdot 100 \quad (1)$$

## 2.3. Rheological properties

Rheological tests were performed using a rotational rheometer (AR-1500, TA instruments) equipped with cone-plate geometry (SN982907, TA instrument, angle = 1.023°,  $\emptyset = 20$  mm, geometry gap = 32  $\mu$ m). First, the linear viscoelastic region (LVR) at 20 and 37 °C was identified by applying a strain sweep between 0.1 and 100%, frequency 1 Hz. Subsequently, temperature sweep tests were performed on hydrogels ( $n = 3$ ) applying a temperature ramp at 2 °C min<sup>-1</sup> between 10 and 60 °C, 0.5% strain (*i.e.*, LVR), frequency 1 Hz. The LCST was identified from storage modulus ( $G'$ ) and complex viscosity ( $\eta^*$ ) curves as the intersection between the interpolant of the initial T range, characterized by a slight decrease of the considered parameter, and the interpolant of the following T range, characterized by an increase of the parameter (Fig. 2b).

## 2.4. UV spectroscopy

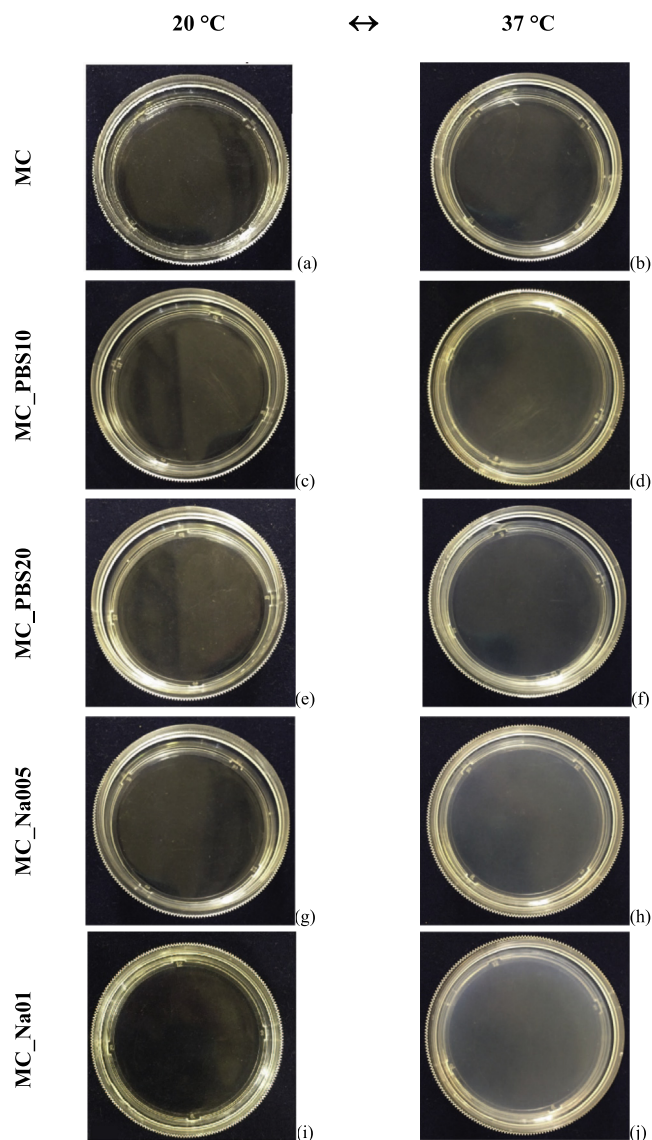
UV–VIS spectroscopy was performed using a spectrophotometer (6705 UV–vis Spectrophotometer, Jenway), by measuring the hydrogels ( $n = 3$ ) absorbance ( $A$ ) at 500 nm and by increasing the temperature between 20 and 50 °C, at 5 °C discrete steps. The percentage of transmittance ( $T\%$ ) was reported in function of temperature following (2), being  $A$  the measured absorbance at 500 nm, and the LCST was identified as the intersection between the interpolant in the initial T region, with stable  $T\%$  values, and the interpolant of the following range, characterized by a decrease of  $T\%$  (Fig. 3b)

$$T\% = \frac{1}{10^A} \cdot 100 \quad (2)$$

All data are reported as mean ± standard deviation. Statistical analysis was performed by ANOVA, considering  $p < 0.05$  statistically significant.

## 3. Results and discussion

Hydrogels  $\Delta W\%$  ranged from 150% for hydrogels produced with lower salts concentration (*i.e.*, MC\_Na01 and MC\_PBS20) to more than 200% for hydrogels produced with higher salts concentration (*i.e.*, MC\_Na005 and MC\_PBS10); all hydrogels showed stable  $\Delta W\%$  values for more than 5 days. Macroscopic images of MC hydrogels are shown in Fig. 1. Hydrogels showed no macroscopic differences at 20 °C (Fig. 1a–e–g–i); in fact, MC is soluble in water at  $T < \text{LCST}$ , due to the formation of hydrogen bonds between water molecules



**Fig. 1.** Macroscopic observation of methylcellulose hydrogels (a–b), MC\_PBS10 (c–d), MC\_PBS20 (e–f), MC\_Na005 (g–h) and MC\_Na01 (i–j) at 20 and 37 °C respectively.

and the MC polymer chains, thus giving a transparent hydrated low viscous hydrogel. Oppositely, hydrogels conditioned for 1 h at 37 °C showed macroscopic differences when observing MC\_PBS20, MC\_Na005 and MC\_Na01 (Fig. 1f–h–j, respectively), which appear opaque due to the sol–gel transition of the hydrogel (*i.e.*,  $T > \text{LCST}$ ) and the hydrophobicity caused by interactions between the MC polymer chains and separation from the water molecules.

The LVR was identified until 50% strain at 20 °C and until 1% strain at 37 °C (data not shown). Temperature sweep tests showed similar rheological trends for all the tested hydrogels (Fig. 2a): in the initial temperature interval,  $G'$  and  $\eta^*$  slightly decrease until

Download English Version:

<https://daneshyari.com/en/article/5463272>

Download Persian Version:

<https://daneshyari.com/article/5463272>

[Daneshyari.com](https://daneshyari.com)