Supporting Information for

Readily Adsorbable Thermoresponsive Polymers for the Preparation of Smart Cell-Culturing Surfaces on Site

Mattia Sponchioni[1,*], *Nicolò Manfredini*[1], *Arianna Zanoni*[1], *Ernesto Scibona*[2], *Massimo Morbidelli*[2], *and Davide Moscatelli*[1]

[1]Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, via Mancinelli 7, 2013 Milano – Italy.

[2]Department of Chemistry and Applied Biosciences, Institute for Chemical and Bioengineering, ETH Zurich, Vladimir-Prelog-Weg 1, 8093 Zurich – Switzerland.

*Correspondence to Mattia Sponchioni, E-mail: mattia.sponchioni@polimi.it

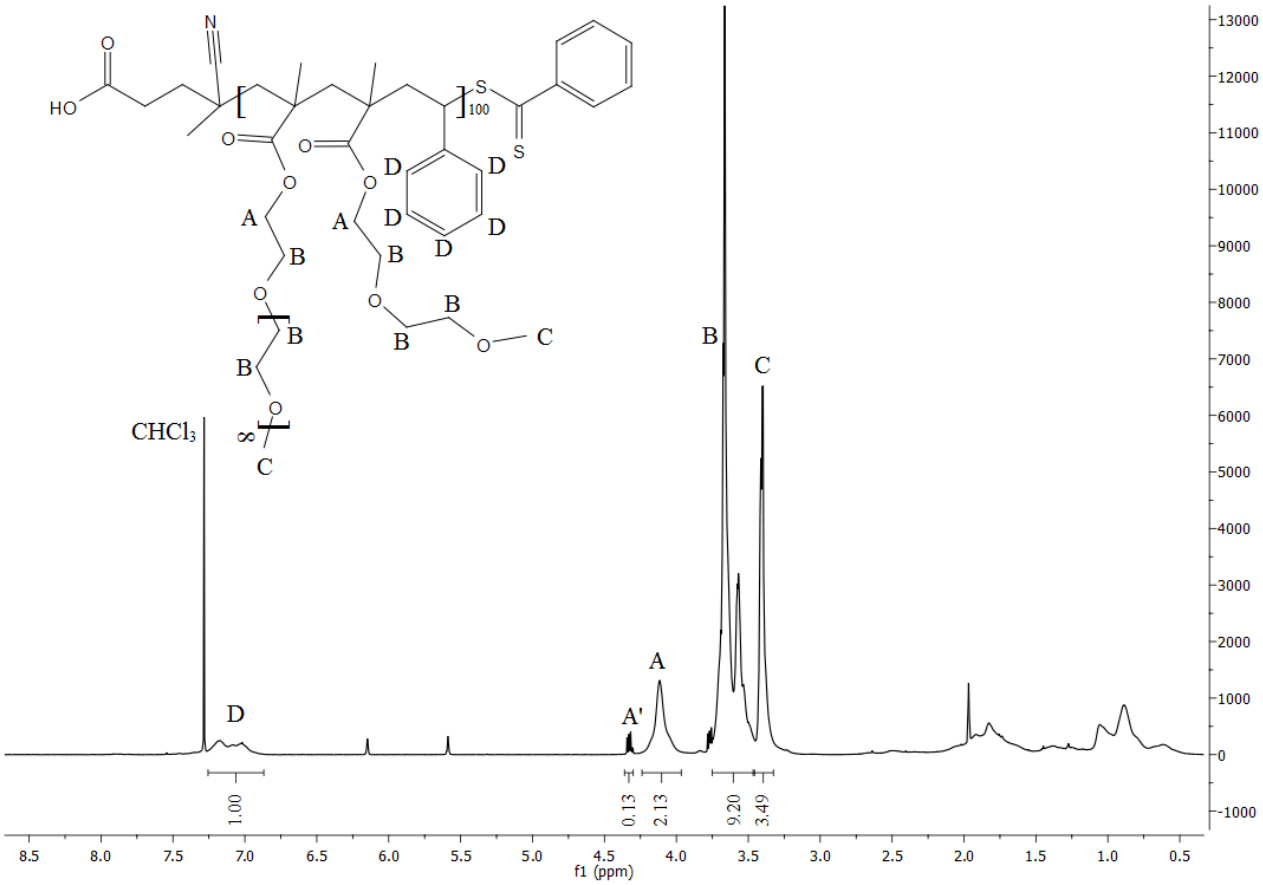

Figure S1. ^{1}H NMR spectrum of the statistical copolymer Stat10 recorded in $CDCl_3$ on a Bruker 400 MHz spectrometer.

From the copolymer ^{1}H NMR spectrum reported in **Figure S1**, the EGnMA (*i.e.* EG_8MA+EG_2MA) conversion can be calculated according to the following **Equation S1**.

$$\chi_{EGnMA} = \frac{A}{A + A'} \tag{S1}$$

Being A the signal of the protons close to the methacrylate group in the copolymer chain and A' the signal generated by the same protons in the unreacted monomer. In all of the cases, high monomer conversion (*i.e.* >93%) is obtained, thus confirming that the RAFT polymerization occurred properly. On the other hand, the styrene mole fraction ($x_{Styrene}$) and the EG_8MA mole fraction (x_{EG8MA}) in the copolymer chains can be determined according to **Equation S2** and **S3**, respectively.

$$x_{Styrene} = \frac{\frac{D}{5}}{\frac{D}{5} + \frac{A}{2}} \tag{S2}$$

$$x_{EG_8MA} = \frac{\frac{A}{2}}{\frac{D}{5} + \frac{A}{2}} \frac{\frac{2B}{A} - 6}{26} \quad \text{(S3)}$$

Where D is the signal generated by the aromatic protons in the styrene and B that generated by the protons in the ethylene glycol units.

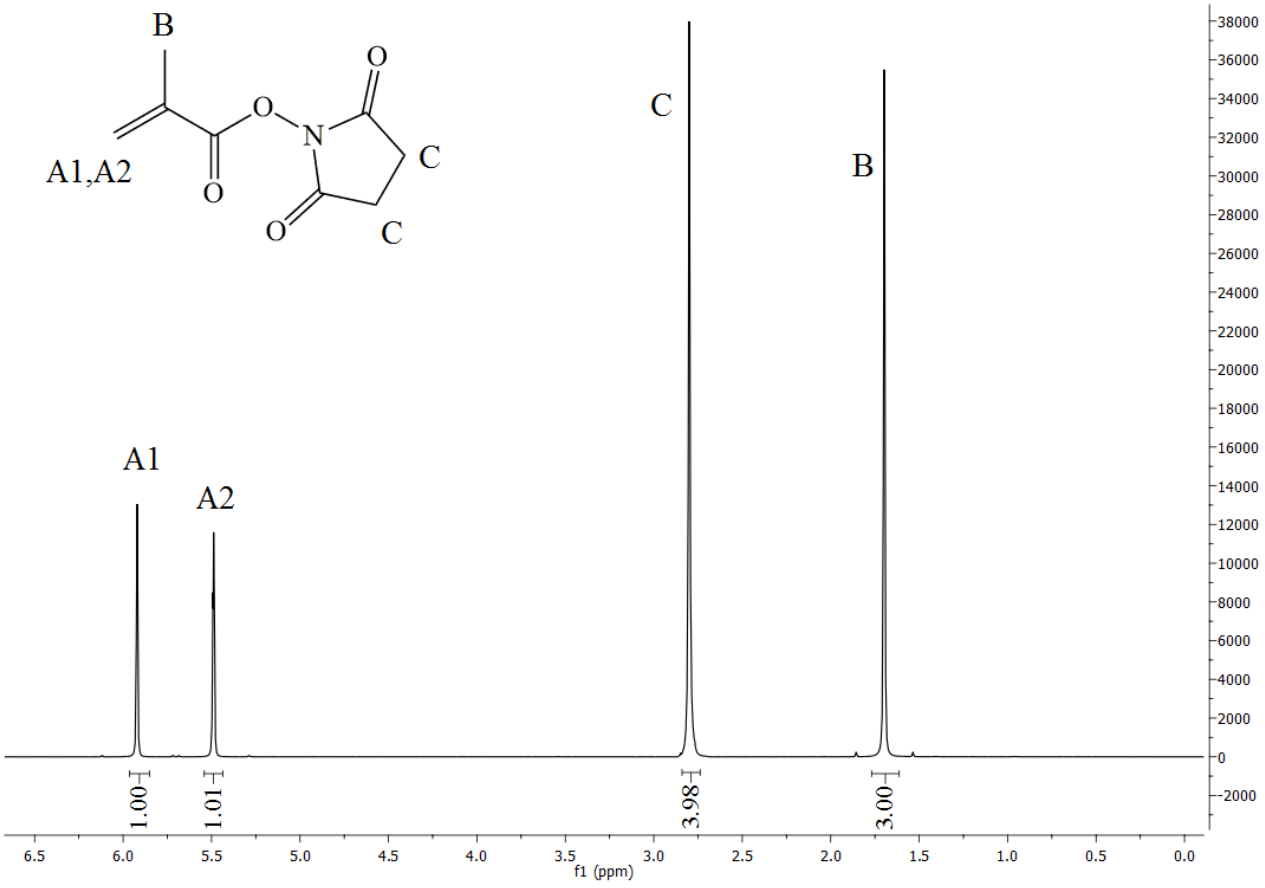

Figure S2. ^{1}H NMR spectrum of the methacrylic acid-NHS adduct recorded in $CDCl_3$ on a Bruker 400 MHz spectrometer.

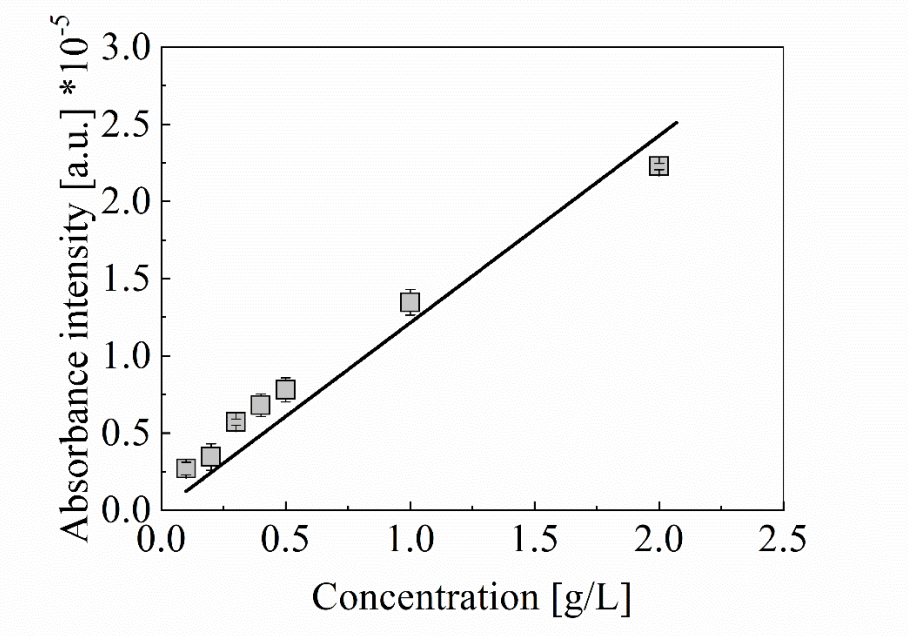

Figure S3. Calibration curve obtained for the sample Stat10, reported as an example. This calibration curve was obtained by measuring the absorbance at a wavelength of 450 nm, for polymer solutions at different concentrations. The same procedure was applied to all of the samples synthesized.

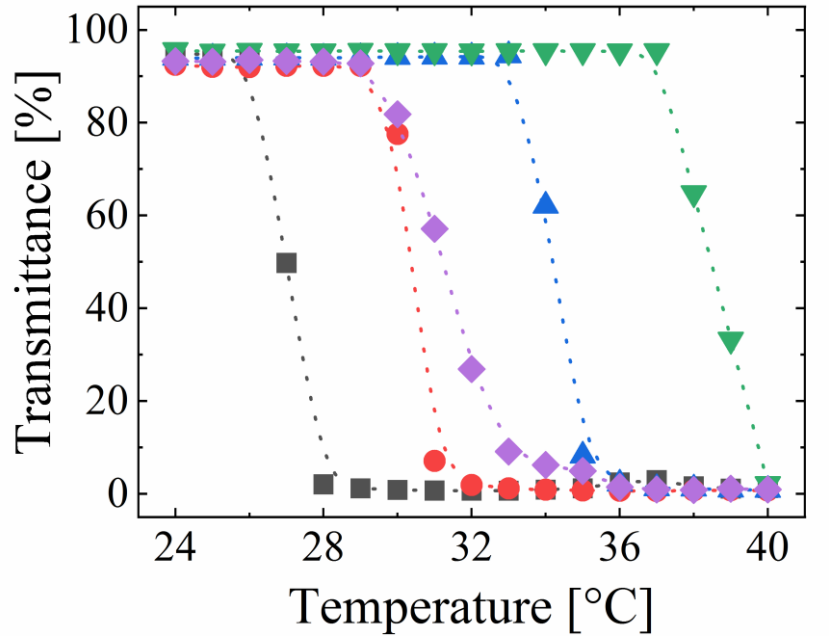

Figure S4. Transmittance of 2 mg/mL polymer solutions in PBS as a function of temperature for Stat5 (■), Stat10 (●), Stat15 (▲), Stat20 (▼) and Block (♦). The transmittance was measured at 500 nm with a temperature increment of 1 °C after each measurement and 10 min as equilibration time. The Tcp reported in Figure 2b is calculated as the inflection point of the sigmoidal curves transmittance vs. temperature.

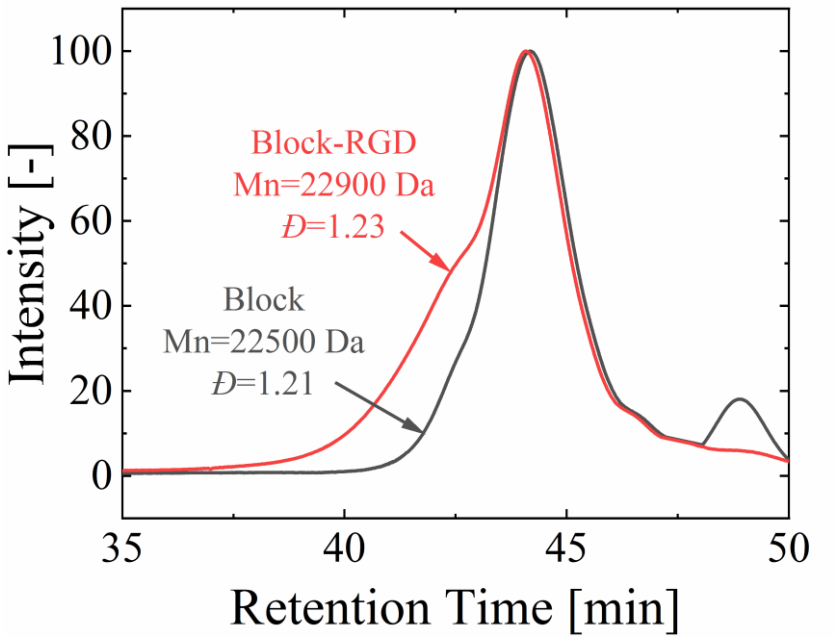

Figure S5. GPC chromatograms for the samples Block and the corresponding Block-RGD, obtained from the functionalization of the former with the RGD motif. The successful grafting is demonstrated by the higher molecular weight of Block-RGD.

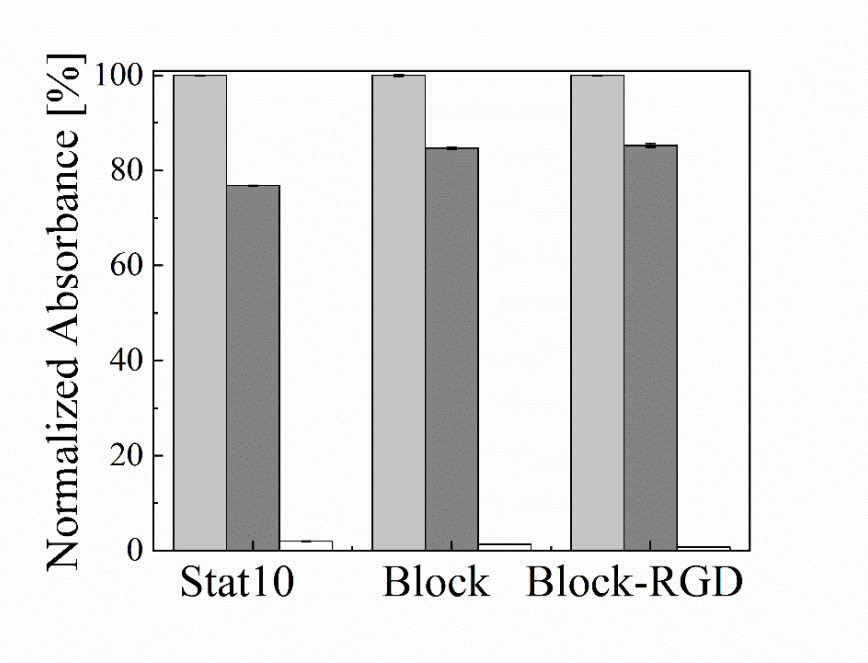

Figure S6. Normalized absorbance for polymer solutions measured at 450 nm before (light grey bars) and after (grey bars) the adsorption on TCPS surfaces as well as for the water used for rinsing the surface (white bars).

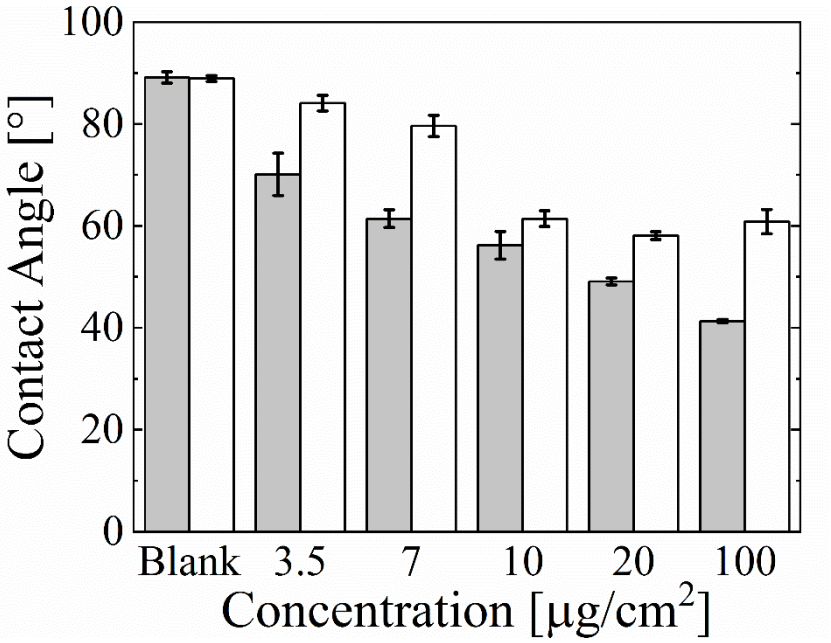

Figure S7. Influence of the copolymer density over the contact angle of the surface below (grey bars) and above (white bars) the Tcp in the case of Block. The contact angle was measured using PBS supplemented with 10% FBS to mimic the cell culturing medium.

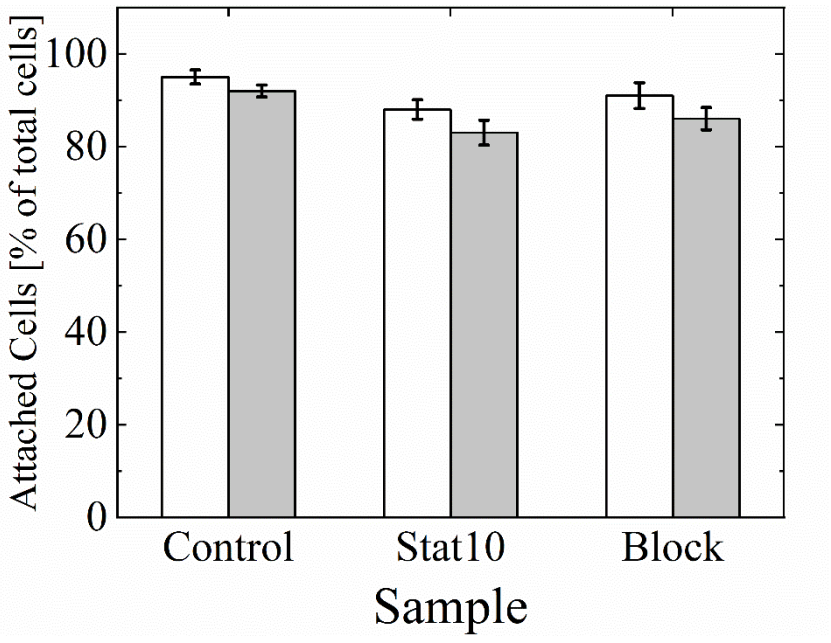

Figure S8. Initial attachment, after 24 h, of CHO cells (white bars) and ASC (grey bars) to thermoresponsive coatings compared to the bare TCPS surface as control.

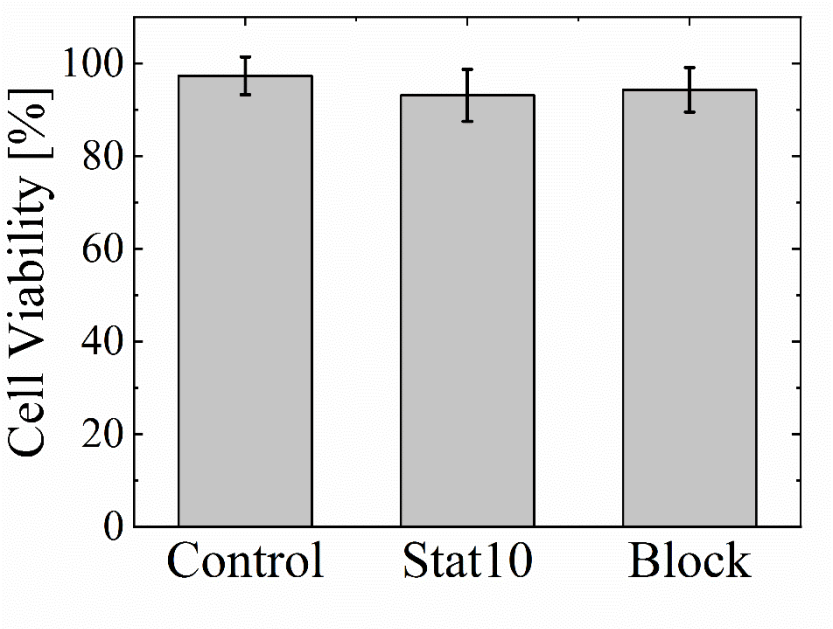

Figure S8. ASC cell viability measured *via* Trypan Blue staining and expressed in terms of percentage of alive cells with respect to the total number of cells. In the cases of Stat10 and Block, the cells were seeded on TCPS surfaces coated with the corresponding thermoresponsive polymer at 30000 cells/ml and incubated at 37 °C for 48 h. Experiments were conducted in triplicate.

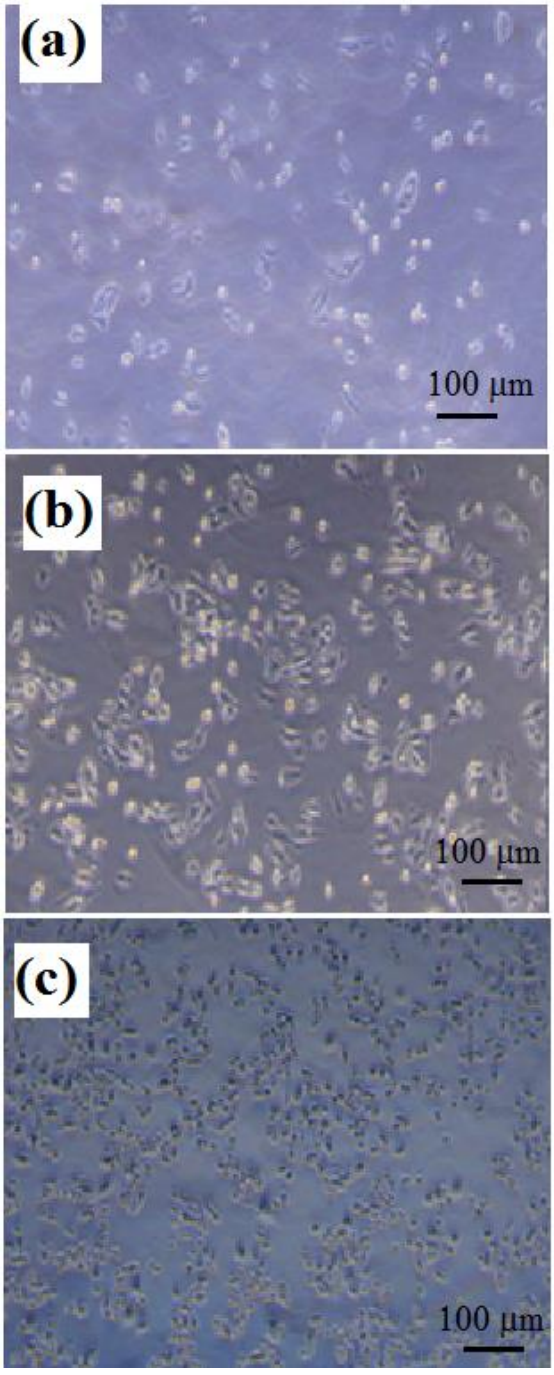

Figure S9. CHO cultured on thermoresponsive surfaces (Block, see Table 1): (a) seeding at 15000 cells/cm^2, detachment by incubation at 25 °C for (b) 30 min and (c) 60 min. Magnification = 400 x.