Electronic Supplementary Material (ESI) for Biomaterials Science.
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Supplementary information

Diffusion of reactive species were analyzed by mixing 500 µL of Griess Reagent with 500uL of methylcellulose (MC) prior plasma treatment. As observed in Figure S1, the diffusion of reactive species clearly improved when the sample was magnetically stirred during treatment.

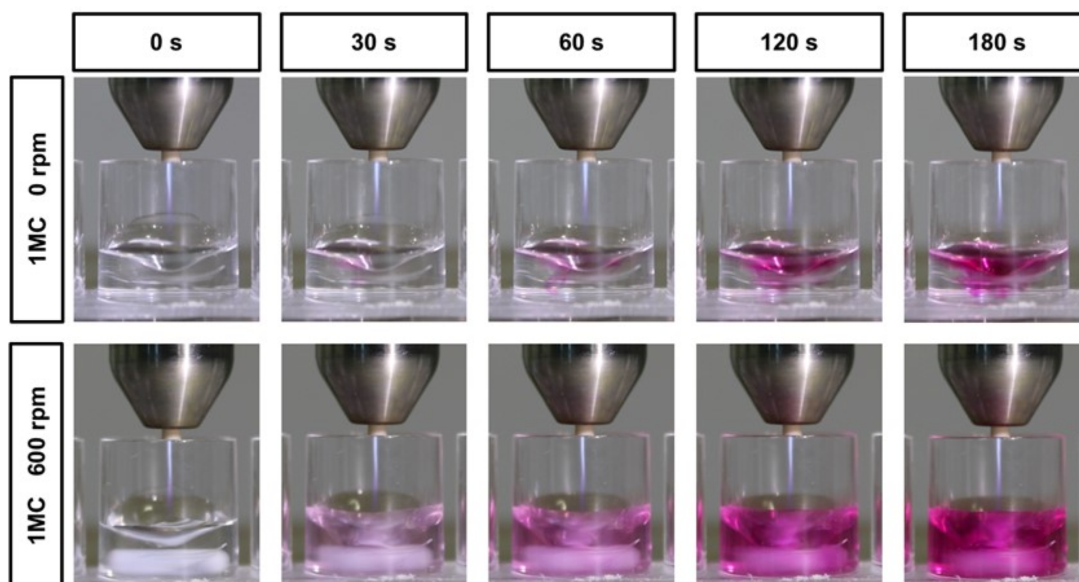


Figure S1. Images showing the diffusion of reactive species with and without magnetic stirring (top and bottom images, respectively).

Figure S2 shows the SEC chromatograms of pristine and plasma-treated 1MC. Note that samples were diluted after the plasma-treatment, not before. As observed, the peak attributed to the elution of MC did not shift to higher elution times. Plasma treatment could only break up a small proportion of MC polymeric chains and was only observed on the diluted samples. This means that the modifications on molecular weight of plasma treatment are covered due to high concentration of non-modified MC.

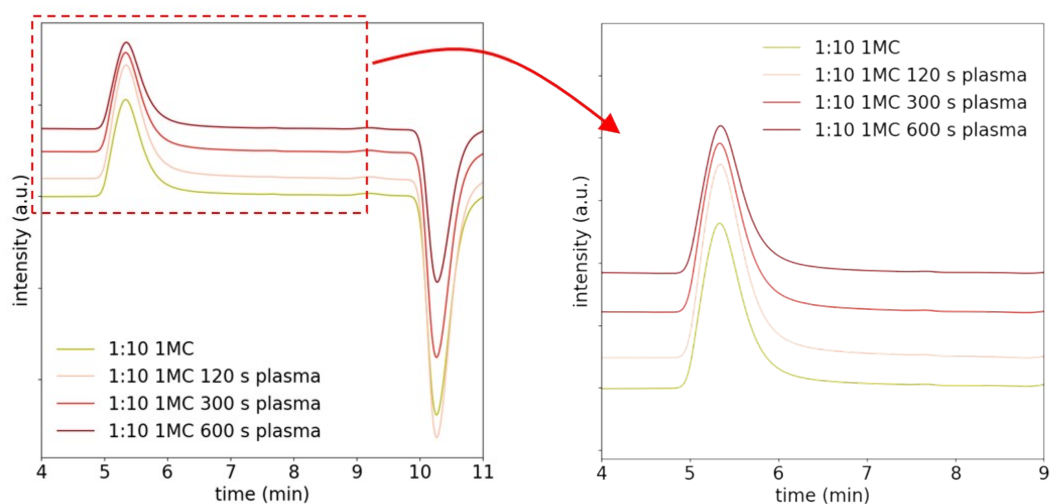


Figure S2. Analysis by size exclusion chromatography of plasma-treated MC solutions. Note that the dilution was conducted after plasma-treatment.

Different concentrations of H_2O_2 were prepared (from a 30 wt.% solution) in 3.5HP and subsequently stored for 24 h at 37ºC. The concentration of H_2O_2 was quantified before and after the incubation in order to study the influence of the incubation on the stability of H_2O_2. As observed in Figure S3, the concentration of H_2O_2 remained constant after incubation. Therefore, the temperature by itself did not have an influence over the stability of H_2O_2.

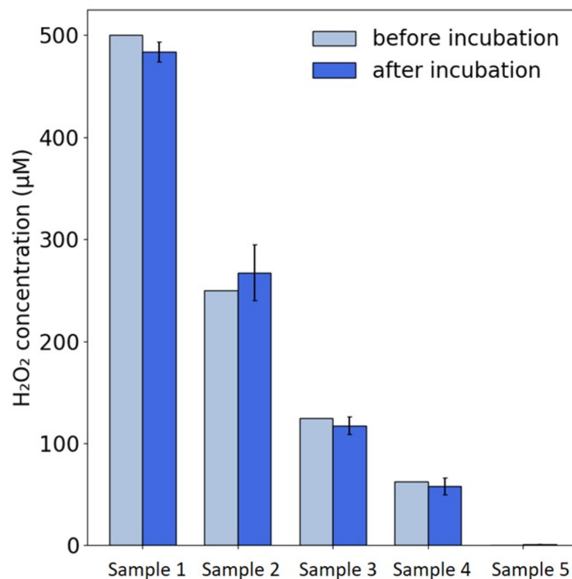


Figure S3. Concentration of H_2O_2 in 3.5HP before and after incubation at 37ºC for 24h.

The chemical probe coumarin (COU) (Sigma Aldrich, USA) was employed to detect OH radicals. Different dilutions of 1MC3.5HP (1:10,1:100,1:1000) were prepared in 1mM COU, and different plasma-treatment times were evaluated. In solution, OH radicals react with COU giving a fluorescent product: 7-hydroxcoumarin (hCOU). The fluorescence intensity of 500 µL of plasma treated MC solutions were measured with a Synergy™ HTX Multi-Mode Microplate Reader ($\lambda_{ex/em}$ = 360/460).

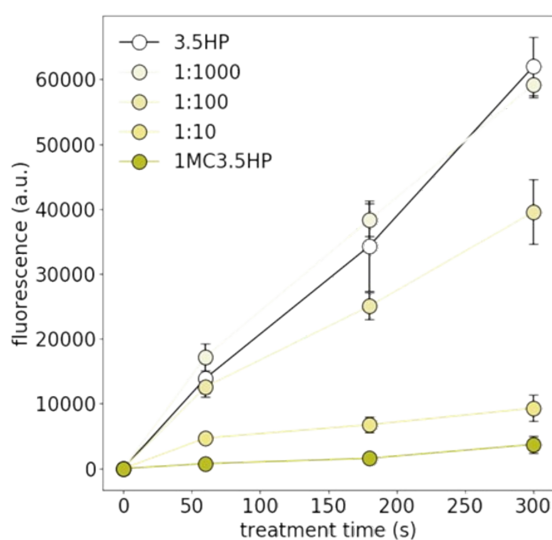
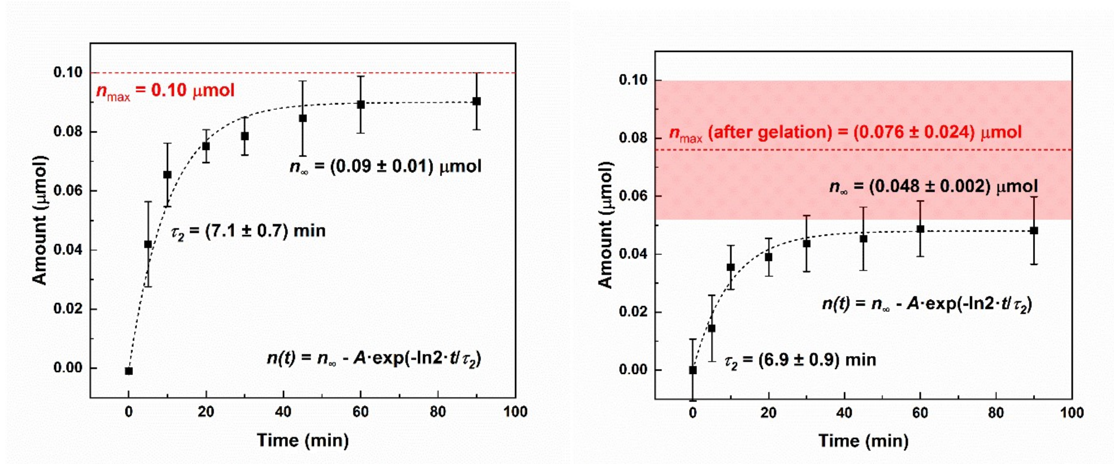


Figure S4. hCOU fluorescence after plasma treatment at different MC dilutions

Figure S5 presents the release data of nitrite ions and peroxides added to the hydrogel from standard solutions.



(a) (b)

Figure S5. Moles of nitrite ions (a) and of hydrogen peroxide (b) released from a 200 µL 1MC3.5HP hydrogel in PBS as release media at 37 °C as function of the release time, quantified by colorimetric probes. Nitrite and hydrogen peroxide were added to the hydrogel from standard solutions. The data are the average of three replicates ± standard deviation. The black dashed line is the exponential fit of the experimental data that allowed to obtain a good estimate of the time constant of the process and the red dashed line represent the total amount of RONS in the hydrogel before starting the release experiment.

The data in the figure have been interpolated using an exponential model:

$$n(t) = n_\infty - Ae^{\frac{-\ln 2 \cdot t}{\tau_2}}$$

where n_∞ is the amount in moles released at infinite release time, A and τ_2 are respectively the amplitude and the doubling time of the exponential. From these data we can conclude that the RONS are released from the hydrogel by simple diffusion mechanisms with time constants that do not depend on the nature of the specific species and are of the order of 7 min. According to these results, the total release is achieved within 1 hour.