Responsive Colloidal Dispersions for Reversible Building and Deconstruction of 3D Cell Environments

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Supplementary information

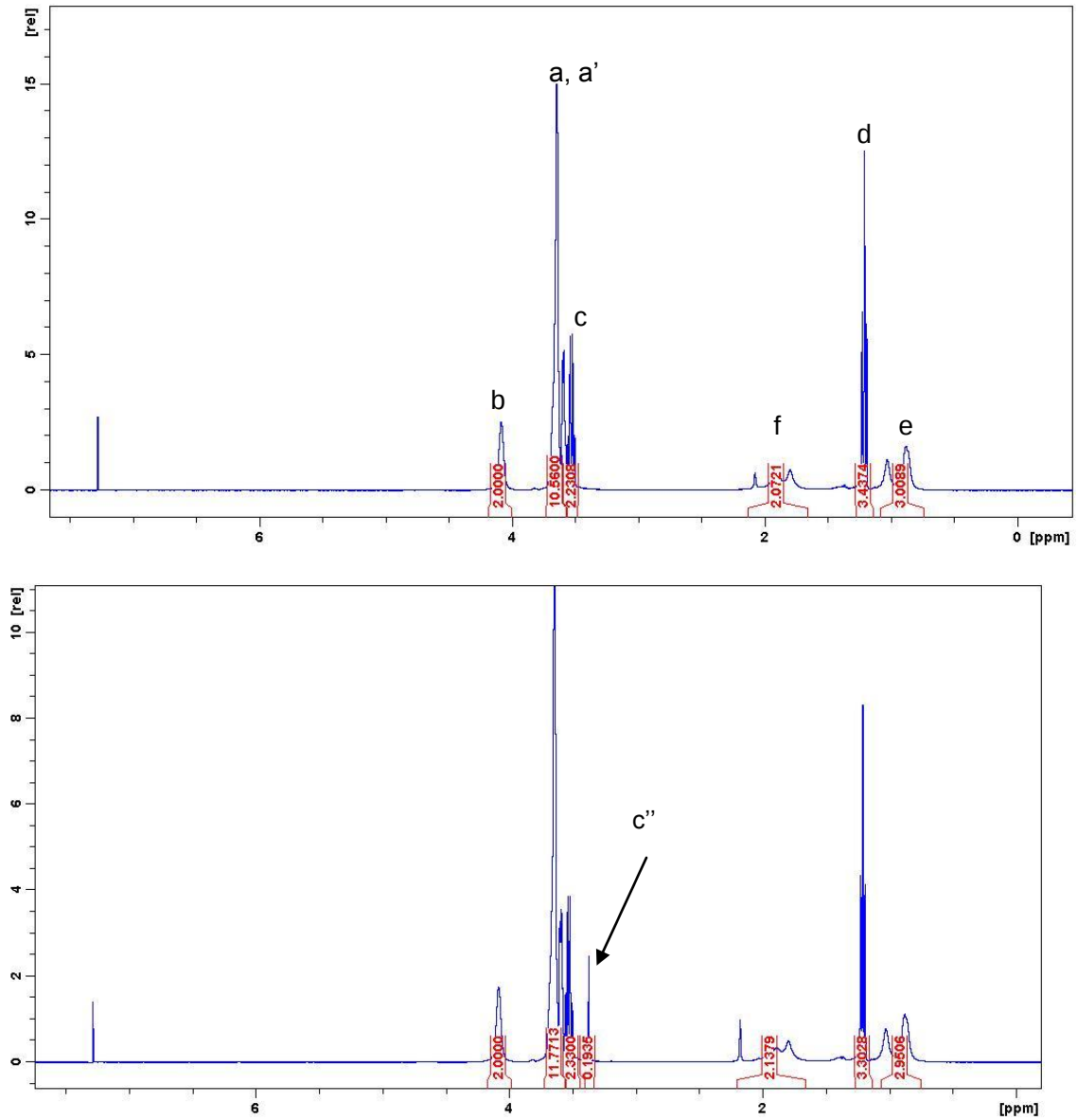


Figure S 1: NMR spectra of PEGMA-EE_{246} (top) and PEGMA-EE_{246}-co-PEGMA-ME_{475} (bottom)

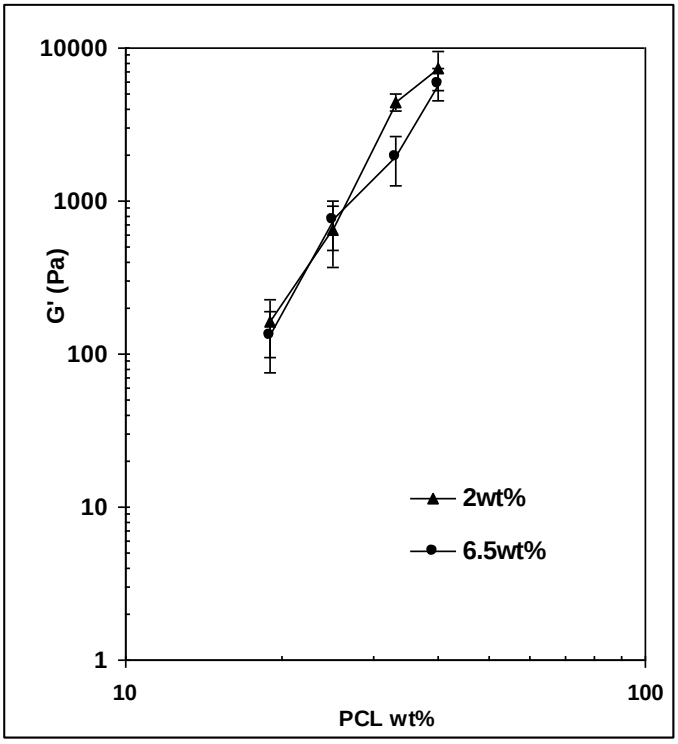


Figure S 2: Effect of increasing PEGM-EE246 on gel strength. Above 2 wt% PEGMA-EE246 in the gels there is no further increase in G'

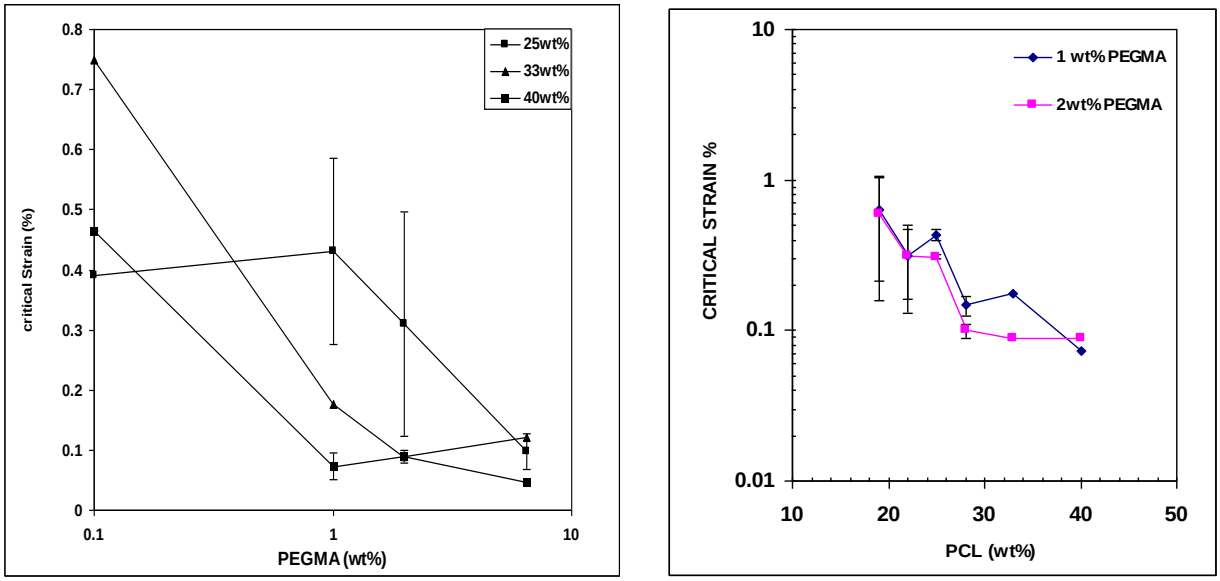


Figure S 3: A general trend of increase in gel brittleness with increasing PEGMA content and PCL content. Increase the % content of the 2 components in gels give stronger but more brittle gels

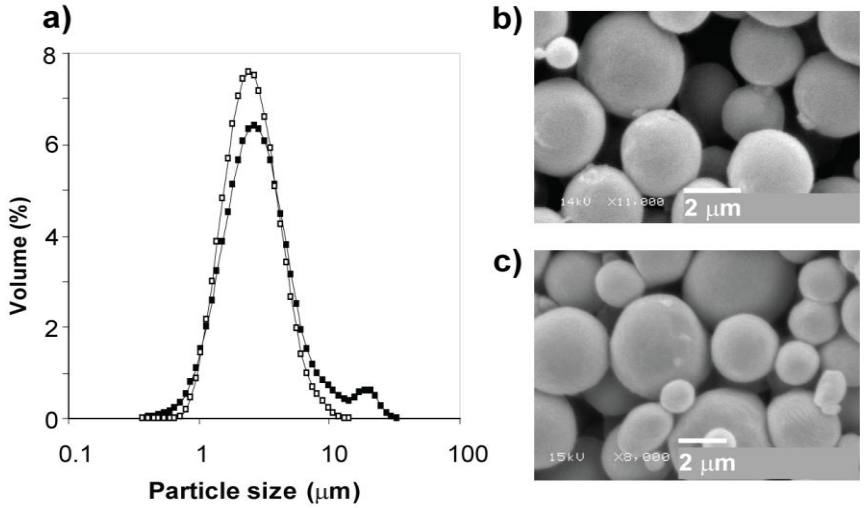


Figure S4 The size distribution (volume %) of the polycaprolactone microparticles before (closed symbols) and after (open symbols) NaOH treatment. The morphology of the microparticles as it appears by SEM before (top) and after (bottom) NaOH treatment.

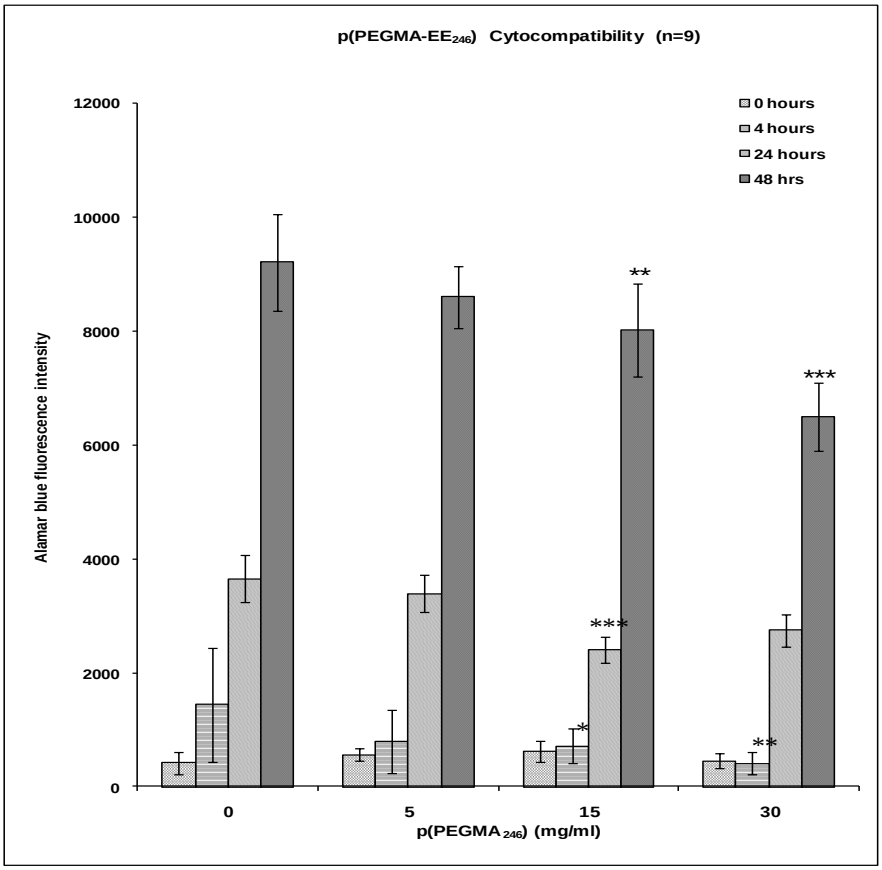


Figure S 5: Effect of p ($PEGMA_{246}$) on NIH 3T3 mouse fibroblasts viability and proliferation.3T3 mouse fibroblasts were of passage 27. The cells metabolic activity, was assessed using Alamar blue, at specific time points from adding increasing amounts of the polymer p($PEGMA\text{-}EE_{246}$). The polymer was added after the cells were allowed to attach to the tissue culture plastic for 4 hours, and an Alamar blue assay was performed immediately (0 hours time point), followed by other measurements after 4 hours, 24 hours and 48 hours of incubation with the polymer.

No statistically significant drop in cell metabolic activity were detected until p(PEGMA-EE246) concentrations reached 15mg/ml. Although this can indicate some cell loss, the remaining cells were able to proliferate normally in the presence PEGMA at all concentrations tested (values are means± S.D, n=3). A t-test was performed to identify significant differences between the control and the samples (* refers to $p<0.05$, ** to $p<0.01$ and *** to $p> 0.001$ respectively)

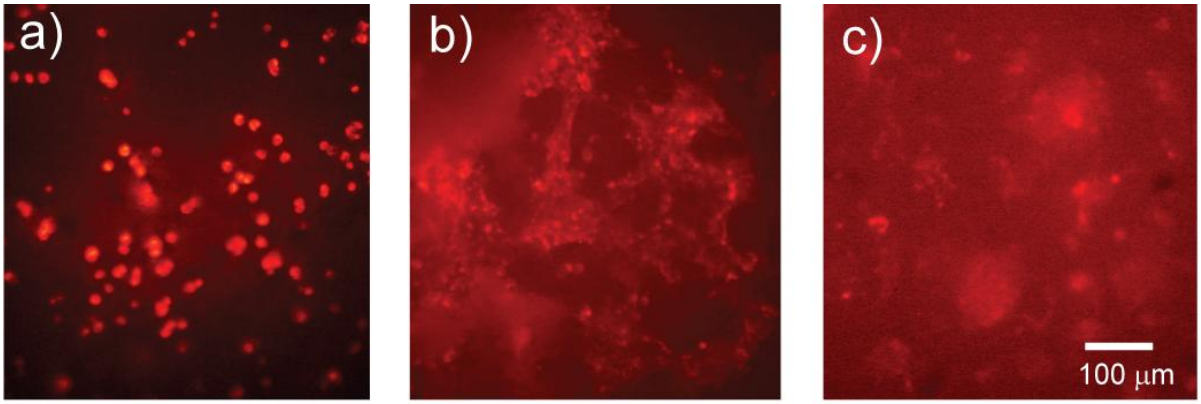


Figure S 6: Fluorescence images of NIH 3T3 cells cultured on top of the gels after a) Day ; b) Day 5 and c) Day 8. Cells were labelled with Cell-Tracker OrangeTM . The cells attached to the colloidal gels surface with a mostly rounded morphology on day 1, but spread significantly and secreted ECM after that, with most of the surface being covered by cells on day 8, as indicated by the strong fluorescence signal throughput the gel. Scale bar: 100μm

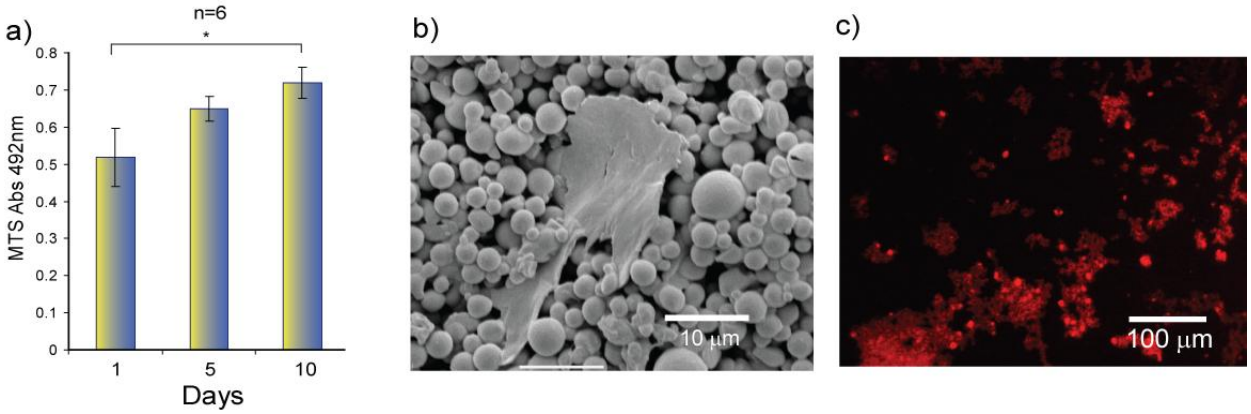
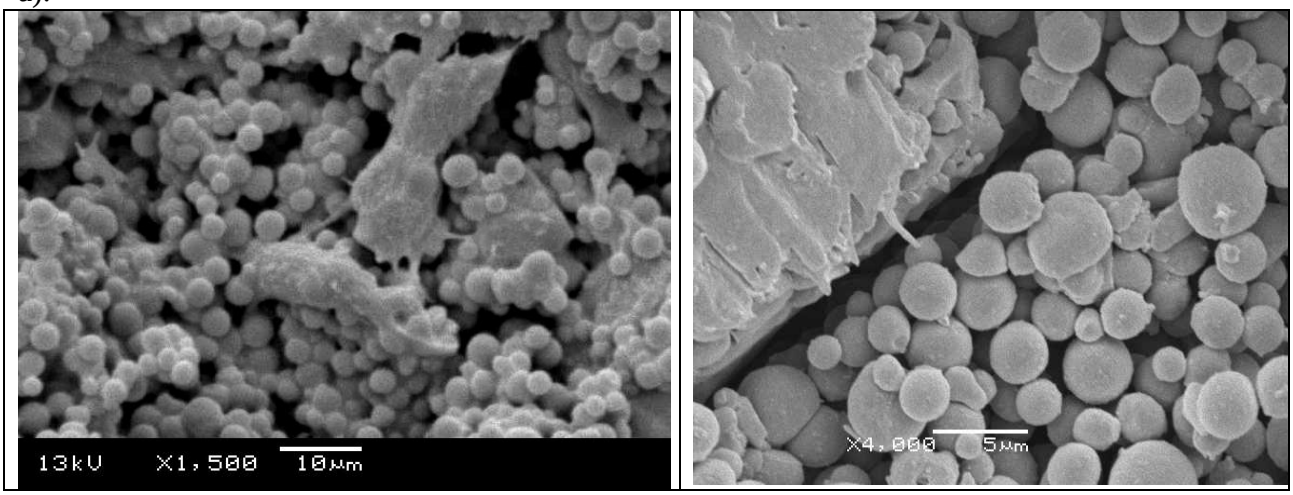
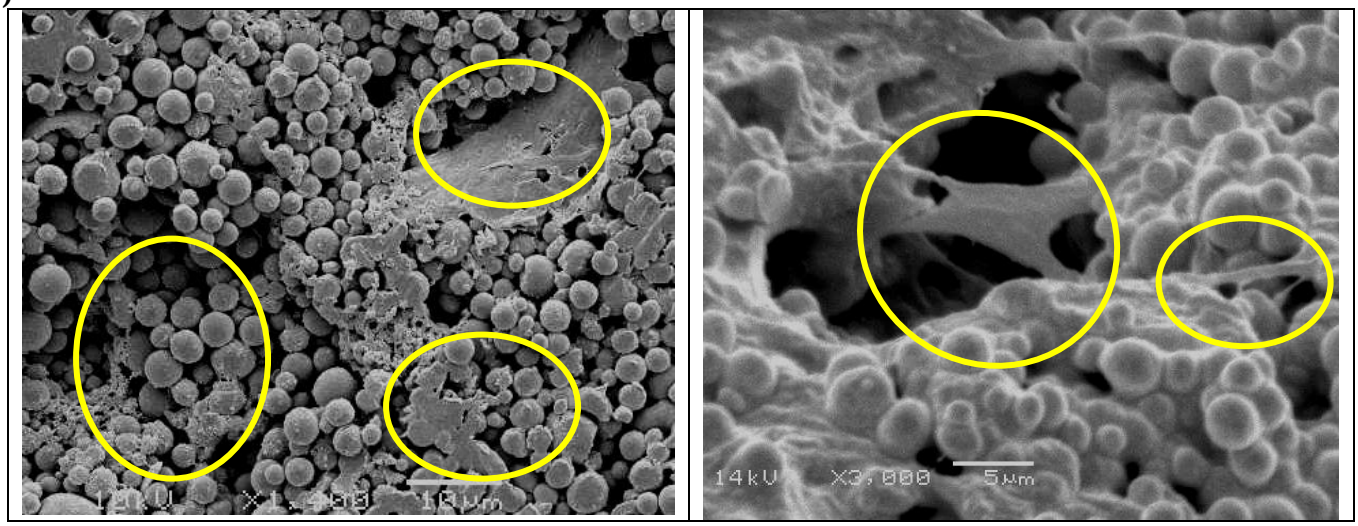


Figure S 7 Culture of Swiss albino 3T3 fibroblasts within the gels. The metabolic activity of the cells was assessed by MTS assays (a . The increase in released MTS dye indicated proliferation; b) SEM images of the cells within the gels at the end of culture, indicating spreading and interactions with the PCL particles; c) Microscopyof cell particles aggregates, obtained by diluting a gel in which cells were seeded and incubated at 37°C for several hours. Cells were labelled with Cell-Tracker Orange™. The fractal structure of the particles flocs is apparent and cells can be seen interacting with particles. Scale bar =100μm

a).



b)



c)

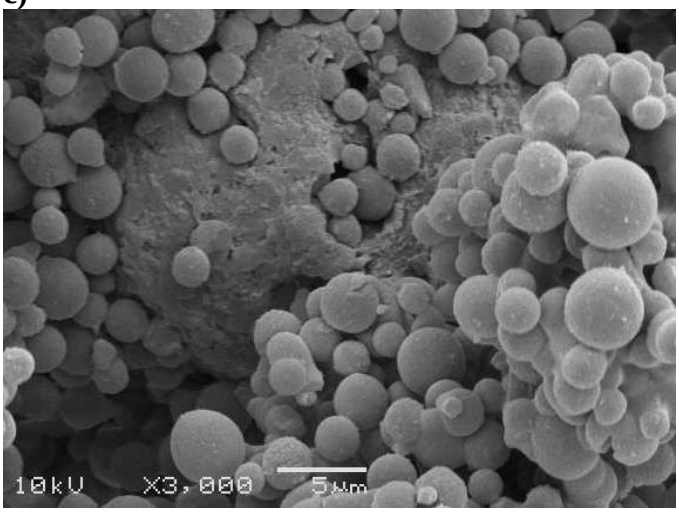


Figure S 8: SEM images capturing different behaviours of NIH3T3 cultured in the gels.

A) NIH 3T3 cells interaction with the particles gels, with lamellipodia apparent. projecting from the cells. B). ECM secretion in the gels by NIH3T3 cells cultured in the gels for 1 week (*left*) and after a month (*right*). Areas of suspected ECM are circled. C) NIH 3T3 cell in the middle of division after 3 days of culture in the gels.